AN INVESTIGATION OF THE EFFECTS OF ELEMENTAL DIET FEEDING ON BILE ACID METABOLISM AND SMALL INTESTINAL STRUCTURE AND FUNCTION

LESLEY MARGARET NELSON B.Sc. (Hons.)

(Gastroenterology Unit, Glasgow Royal Infirmary)

Thesis submitted for the degree of
Doctor of Philosophy in the
Faculty of Medicine, University of Glasgow

October 1979

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ACKNOWLEDGEMENTS

My sincere thanks are due to Professor E.M. McGirr of the Department of Medicine for providing excellent facilities and who supervised this project, and to Professor H.G. Morgan of the Department of Pathological Biochemistry who acted as co-supervisor.

I am particularly indebted to Dr. R. I. Russell, Consultant-in-Charge of the Gastroenterology Unit, Glasgow Royal Infirmary, wherein I was employed as a Clinical Biochemist, and who was instrumental in initiating the project. He undertook daily supervision of the work and his frequent discussions were greatly stimulating.

Dr. F.D. Lee of the Department of Pathology kindly provided facilities and assistance for the studies of the morphology of rat jejunum.

I would also like to thank Miss H. Fleming for skilled technical assistance, Mr. F. Speirs for assistance with the illustrations and Mrs. Jane Dick for her patience while typing the thesis.

Publications Arising from Thesis

Papers

1. The use of an elemental diet (Vivonex) in the management of bile acid induced diarrhoea.
   L. Nelson, H.A. Carmichael, R.I. Russell and S.T. Atherton
   Gut 18, 792-794 (1977)

2. Small intestinal changes induced by an elemental diet (Vivonex) in normal rats.
   L.M. Nelson, H.A. Carmichael, R.I. Russell and F.D. Lee
   Clinical Science and Molecular Medicine 55, 509-511 (1978)

Abstracts

1. *The use of an elemental diet (Vivonex) in the management of bile acid induced diarrhoea.
   L. Nelson, H.A. Carmichael, S.T. Atherton, R.I. Russell
   Gut 17, 387-388 (1976)

2. *The management of bile acid induced diarrhoea by the elemental diet Vivonex.
   L.M. Nelson, H.A. Carmichael, S.T. Atherton, and R.I. Russell
   Scottish Medical Journal 22, 108 (1977)

3. *Small intestinal changes induced by an elemental diet (Vivonex) in rats.
   L.M. Nelson, H.A. Carmichael, R.I. Russell, and F.D. Lee
   Gut 18, 945 (1977)

4. The effect of an elemental diet (Vivonex) on exocrine pancreatic secretion in man.
   S.T. Atherton, L.M. Nelson, E. Robertson, R.I. Russell
   Scottish Medical Journal 23, 115 (1978)

* Personally presented.

TABLE OF CONTENTS

Page

TABLES AND ILLUSTRATIONS

SUMMARY

The work reported in this thesis examines the effect of commercially available 'elemental'diets on bile acid metabolism and small bowel structure and function in rats and humans.

The development of elemental or synthetically formulated, chemically defined diets is described in Chapter 2, and bile acid metabolism and gut structure and function are discussed in Chapters 3 and 4 of the introductory section, Section I. In Section II the results of the studies on the effects of elemental diets on bile acid metabolism and pancreatic secretion are reported and in Section III small bowel structure and function in relation to elemental diet feeding have been investigated. The two diets studied were Vivonex and Flexical, which differ in their fat content, the nature of the nitrogen source and the carbohydrate composition. Section IV is a general discussion section.

Section II

Measurement of faecal bile acids using gas liquid chromatography is discussed in Chapter 5 and the method used for the experiments in Chapters 6, 7 and 9 is described. After extraction and deconjugation, the bile acids were chromatographed as the methyl esters on OV-17 columns employing 23-Nordeoxycholic acid as a true internal standard throughout the method. The coefficient of a variation for duplicate analyses in the same batch was 11.4% and between batches it was 12.8%. This was considered to be satisfactory for a long complicated assay, and for the study of gross changes induced by elemental diet feeding.

The effect of elemental diet feeding for three months on rat body weight, faecal weight and total faecal bile excretion is described in Chapter 6. An initial lag was noted in body weight gain for the rats fed Vivonex but the body weight of the Vivonex fed rats was in fact greater than that of the controls at the end of the study, although the difference was statistically significant for only two of the three groups studied. Flexical fed rats gained more weight than controls but the difference was only statistically significant at weeks 3 and 8. Dry weight of faeces was markedly reduced in the elemental diet fed rats from about 50g/week to 2 or 3g/week. Total faecal bile acids were significantly reduced in the rats fed Vivonex compared with controls in all three groups studied. Similar results were obtained for Flexical feeding suggesting that the very low fat content of Vivonex is not its most important property in this respect.

In Chapter 7 the composition of the bile acids excreted was investigated and a reduced bacterial degredation found in the rats fed Vivonex. The changes with Flexical feeding were somewhat different and did not suggest the same reduction of bacterial degredation as with Vivonex feeding.

In Chapter 3 bile acid kinetics were studied in the rats by following the excretion of ^{14}C labelled cholic acid. A marked reduction in bile acid turnover, but no change in the calculated pool size, was found during elemental diet feeding. Liver cholesterol levels were also measured and found to be elevated in the rats fed the elemental diets. Again the changes were more marked in the rats fed Vivonex.

In Chapter 9 the results of the animal studies have been extended to humans, and the use of Vivonex in the management of patients with bile acid induced 'cholerheic' diarrhoea investigated. A significant reduction in faecal bile acid excretion was found with a concomittant improvement in the diarrhoea of these patients. One possible contributing factor was thought to be reduced gallbladder stimulation as a result of the low fat and low nitrogen content of Vivonex. The effect of Vivonex compared with a Lundh test meal on pancreatic secretion was therefore examined as reported in Chapter 10. A reduced cholecystokinin-pancreozymin response was indicated by the reduced trypsin secretion induced by Vivonex.

Section III

The effects of Vivonex and Flexical feeding on the morphology of rat jejunum were investigated, in Chapter 11, since bile and pancreatic secretions are known to influence small bowel structure and function. Both diets resulted in a reduced crypt height to villus height ratio indicative of an improved survival of the mature enterocyte population, in both the jejunum and ileum. When the effects on jejunum and ileum were compared a slight difference between the two diets was observed. In Chapter 12 alkaline phosphatase and disaccharidase activities of the jejunal mucosa were investigated. Very little functional change accompanying the morphological changes during elemental diet feeding was observed when assessed by this method, and disaccharidase activity may be directly

affected by the carbohydrate content of the diets anyway.

A more physiological measure of function was investigated, as described in Chapter 13, by perfusing the jejunum in vivo, in anaesthetised rats, and measuring water and glucose absorption. The results obtained were rather surprising in that there was no difference between the control and Vivonex fed rats, but the jejunum of the rats fed Flexical absorbed significantly less than the controls. The results are discussed in relation to nutritional demand and humoral regulation of small bowel function, and it is possible that the ileum may in fact show a difference between the control and Vivonex fed rats. The initial results for the morphological changes in rat jejunum during elemental diet feeding suggested that Vivonex might be beneficial in the initial treatment of patients with adult coeliac disease, in whom cell turnover is greatly accelerated, and a study of this was undertaken as described in Chapter 14. No additional benefit of Vivonex over treatment with a gluten-free diet alone was demonstrated by perfusion of the proximal jejunum. It is possible, however, that in the distal small bowel, where the gluten damage is less severe, that Vivonex may have had a demonstrable beneficial effect.

CONCLUSIONS

The mechanism of the changes induced by elemental diet feeding, especially those of Vivonex on bile acid metabolism and small bowel morphology, could be further

investigated by repeating the experiments in germ-free rats. Many aspects of the changes induced by Vivonex feeding in rats reproduce the situation in germ-free rats. No access to a germ-free rat colony was available however.

Investigation of the function of the ileum might show a difference between elemental diet fed rats and controls, and may yield important information about the control of normal small bowel structure and function. The use of Vivonex alone in the initial treatment of adult coeliac disease may produce a more rapid improvement than a gluten-free diet, with or without Vivonex supplements, if the low residue nature of the diet is an important property in relation to small bowel morphology.

CHAPTER 1

AIMS OF STUDY

The work of the Greenstein group in developing a complete water soluble chemically defined diet (Greenstein et al, 1960) and the later studies of Bounous and co-workers with liquid semi-hydrolysed diets (Bounous et al, 1967 a,b) paved the way for the production and marketing of commercial elemental diets. These diets for human therapy provide a defined and constant composition for each product. This offers advantages for quantitative studies in that previous studies from different laboratories employed diets with widely varying compositions and made reproducibility of results difficult.

Clinical studies have shown that the commercial elemental diets are safe for long term nutritional support and their usefulness in many clinical situations has been demonstrated. The basic physiological effects of elemental diet feeding have not been investigated to any great extent and many of the clinical studies reported in the literature have been uncontrolled and retrospective.

Certain observations indicated that the effects of elemental diet feeding on pancreatic function, bile acid and cholesterol metabolism, and small bowel structure and function required investigation:

(i) Serum cholesterol levels and gut microflora populations were reduced by elemental diet feeding and the nature of the carbohydrate source influenced the response (Winitz et al, 1970 a,b).

(ii) Faecal bile acid excretion and the degradation of neutral sterols were reduced in three volunteers, although marked changes in faecal flora were not found in this study (Crowther et al, 1973).

(iii) A protective effect of elemental diet feeding against the intestinal lesions produced by hypovolemic shock and 5-fluorouracil administration was demonstrated (Bounous et al, 1967 a,b; 1971 a,b).

(iv) Benefits of elemental diet feeding in patients with complicated pancreatitis were reported (Voitk et al, 1973 d) but reports of the effects on pancreatic secretion in various species are contradictory. (Ragins et al, 1973; Kelly and Nahrwold, 1976).

The aims of the studies reported in this thesis were to investigate the basic physiological effects of elemental diet feeding on bile acid metabolism, pancreatic secretion and small intestinal structure and function in animals, and to apply the findings to patients when appropriate.

The first elemental diet to be marketed in Britain was Vivonex, which was introduced in 1974, and so studies with this diet were originally undertaken. When Flexical became available the studies were extended to compare this diet with Vivonex.

CHAPTER 2

ELEMENTAL DIETS

DEFINITION

There has been much debate in the literature over the description of water soluble diets which contain protein hydrolysates or synthetic amino acids, together with a simple source of carbohydrate and fat, and which also supply the vitamins and minerals necessary to provide a complete form of nutrition.

The development by Greenstein and his colleagues of enzymatic methods for the production of large quantities of pure L-amino acids in the laboratory led to their extensive study of water soluble diets containing synthetic amino acids (Winitz et al, 1960). They used the term 'chemically defined diet' to describe their formulation which contained synthetic amino acids and vitamins together with glucose and minerals. Separate corn-oil supplements containing the fat soluble vitamins were used for the initial animal diets (Greenstein et al, 1957) and so the diets were not complete and the fat source was not chemically defined but a natural food. They later incorporated the fat soluble vitamins into the diet with aid of Tween 80 and ethyl linoleate was added as the source of essential fatty acid. The result was a nutritionally complete, chemically defined, water soluble diet (Greenstein et al, 1960). The commercially available diets based on their formulation, that is containing synthetic amino acids, cannot really be regarded as chemically defined because corn syrup solids, consisting of glucose and glucose oligosaccharides, are used in place of glucose and safflower oil is used rather than pure linoleate.

In 1967 Bounous and his colleagues coined the term 'elemental diet' for their diet formulation containing hydrolysed protein, soybean oil emulsion, sucrose and the necessary vitamins and salts (Bounous et al, 1967 b). The term 'elemental' was thus used in its wider application implying component or constituent parts, rather than the true scientific meaning, and refers particularly to the protein component of the diet.

Nutritionally complete, water soluble diets containing amino acids or hydrolysed protein, together with other nutrients in an easily digestible form, became commercially available for clinical use at the beginning of the 1970's and since then the term 'elemental diet' has been widely used for their description. It is used to describe diets containing synthetic amino acids (Miller and Taboada, 1975) and also those based on protein hydrolysates which include oligopeptides (Bounous et al, 1974).

In 1975 Russell defined an elemental diet as "a food which contains an elemental protein source, in the form of amino acids with other easily digestible nutrients, minerals, and vitamins added and fat present in very small quantities" (Russell, 1975). Of the two diets available in Britain this definition really only applies to Vivonex and does not cover Flexical which contains oligopeptides and amino acids plus a considerable quantity of fat. Since both diets contain corn syrup solids and natural sources of fat they cannot really be considered as "chemically defined" although this term was applied to Vivonex by Crowther et al in 1973.

A third term which has been applied to these diets is "space diet" (Winitz et al, 1965) and there was much interest in their use as nutrition for man in space. This term, however, does not describe the diet but one application of it, and has not been widely used. The diets have also been described as "synthetic", but again this is not an accurate description of the commercial products and is not used.

The broader definition of an elemental diet as originally coined by Bounous (Bounous et al, 1967 b) thus has greater applicability and covers any water soluble, nutritionally complete diet which contains hydrolysed protein and carbohydrate, regardless of whether the hydrolysis to the constituent monomers is complete or partial.

HISTORY AND DEVELOPMENT

It was demonstrated by Abderhalden and co-workers that dogs could be maintained on hydrolysed protein plus starch, lard and bone ash and he wrote - "We seek proof that the animal organism can wholly cover his needs exclusively with the simplest building units of food" (Abderhalden et al, 1909). He later maintained dogs for 48-74 days on a diet composed of completely hydrolysed casein supplemented with tryptophan together with glucose, fatty acids, bone ash and a mixture of purines and pyrmidines. This led him to state that - "science would at one time make such great advances that the possibility would be afforded to manufacture food-stuffs by synthetic means. Without doubt this objective is presently attainable". (Abderhalden and Hirsch, 1912).

This statement was rather optimistic since the role of vitamins in nutrition did not receive much attention until 1911-1915, and the biochemical techniques for recovering all the amino acids from protein hydrolysates were not available. Although the concept of essential and non-essential amino acids was emerging with the discovery that proline was apparently dispensable from protein hydrolysate and tryptophan wasn't, methionine and threonine were not even identified until 1921 and 1935, respectively. (Greenstein and Winitz, 1961). Thus, attempts to develop synthetic diets became overshadowed by the elucidation of the full amino acid requirements of rats and man, and were not possible until pure L-amino acids could be prepared in quantity as a result of the work of Greenstein and Winitz in the 1950's. (Winitz et al, 1960).

In 1931 Rose began the first of his long series of studies into the amino acid requirements of rats and man. Amino acid mixtures were constructed using some amino acids from protein hydrolysates and synthetic racemic mixtures of others, to imitate the composition of casein as far as it was known. Dextrin, sucrose, salt mixture, agar, lard, cod liver oil and yeast powder were added to the amino acids in the early studies. By successively removing and replacing individual amino acids and monitoring growth the essential amino acids in the rat were identified and the benefit of adding a range of non-essential amino acids to the ten essentials for optimum growth was demonstrated. During the eighteen years of work the diets were modified as crystalline B vitamin preparations became available and

knowledge of the mineral requirements of the rat improved. Celluflour was added to the diets to provide bulk. The results of the work on the amino acid requirements of the rat were summarized in a report by Rose et al, in 1948 (Rose et al, 1948).

The first human experiments were performed by Rose in 1942 in healthy male students. Only articles of food virtually devoid of nitrogen of an unknown nature were used, in addition to the amino acids formulations. These were corn starch, sucrose, butterfat (which had been melted and centrifuged to remove protein) together with inorganic salts, corn-oil and vitamins. All except some of the sucrose and butterfat was baked into wafers and celluflour was used to provide bulk. The remaining sucrose and butterfat were used as a flavouring for the amino acid mixtures together with some lemon juice. Such a diet provided 0.3 - 0.35g of nitrogen per day of an unknown nature. It was discovered that more calories were required for a positive nitrogen balance with amino acids rather than protein at the same nitrogen intake, and 55 Cals/Kg were therefore used in the experiments. Histidine was found not to be essential in adult man, in contrast to the rat, and arginine was also not essential. The daily requirement for each amino acid was derived to the nearest 0.1g by reducing the intake until a negative nitrogen balance was obtained, and then increasing it again to give a positive balance. Daily variations in nitrogen balance and differences between individuals prevented the requirements from being assessed any more accurately (Rose, 1949).

Valine was the final essential amino acid to be quantitated and the 'safe' intake for each amino acid was then derived as twice the top value found as minimal, in three to six subjects, for each amino acid. Histidine and arginine removal from the diet did not cause infertility (Rose et al, 1955). The next report in the series dealt with the role of nitrogen intake. Subjects were fed 'safe' levels of the eight essential amino acids with extra nitrogen as glycine and urea. The original total nitrogen of 10g/day was reduced to 3g/day before a negative balance was encountered. 3.5g/day was the minimal nitrogen intake to give a positive balance. This, however, included unknown nitrogen and possibly unavailable nitrogen from D-isomers to about 0.35g/day (Rose and Wixom, 1955). The work on the amino acid requirements of adult man was later reviewed by Rose (Rose, 1957).

Other workers were investigating the suitability of purified amino acids as a source of nitrogen for the growing rat and obtained growth equivalent to 80-90% of that on whole casein (Rasmara et al, 1949). Reproduction of rats fed protein free rations was demonstrated by Shultze with successful production of the F4 generation. The diets containing 16 amino acids were better than those containing the ten essential's only, but none of the diets used was completely adequate for optimum pre-weaning or post-weaning weight gain, or in preventing the development of fatty liver during lactation (Shultze, 1956). Both these reports indicated that optimum proportions of amino acids, and

possibly vitamin and mineral balances, had not been achieved in the diets. The use of D-L racemates for some of the amino acids also presented problems.

During the 1950's Greenstein and his group developed resolution procedures which permitted the large scale preparation of optically pure amino acids designed to provide the basis for completely synthetic diets for use in quantitative metabolic studies and appropriate for parenteral administration. The enzymatic resolution procedure was based on the chemical formation of an N-acetylated racemic mixture of the amino acid followed by specific enzymatic hydrolysis to the L-amino acid (Winitz et al, 1960).

A water soluble, chemically defined diet was formulated so that quantitative metabolic studies could be undertaken for the first time (Greenstein et al, 1957). The liquid diet at 50% concentration, containing synthetic L-amino acids, glucose, vitamins and the required minerals, allowed intake to be accurately measured. Fat soluble vitamins in corn oil were initially supplied as a supplement but later were incorporated into the diet with the aid of Tween 80, and ethyl linoleate was added as a source of essential fatty acid. The essential amino acids were present in nearly the same relative proportions as recommended by Rose (Rose, 1949) and supplied 9.5g of essential nitrogen to 15.7g of non-essential nitrogen. The composition of the non-essential nitrogen could be varied.

In the first report of the series, the effect of different sources of non-essential nitrogen on rat growth

and reproduction was investigated. Better growth was obtained with the non-essential amino acids modelled on casein rather than muscle or ovalbumin. With just a few non-essential amino acids, or with D-L amino acids supplying the non-essential nitrogen, the growth was also less than with non-essential nitrogen modelled on casein. The effects of the different non-essential nitrogen sources on reproduction were also examined, and smaller litters were found with the diets poor in non-essential nitrogen and no litters were produced when the F1 were mated. The F1 generation from rats fed casein-modelled diets successfully produced litters (Greenstein et al, 1957).

Nitrogen balance studies on similar diets with a good source of non-essential L-amino acids (Diet 26), a few non-essential L-amino acids (Diet 3), and the same amino acids as in Diet 26 but the D-L racemic mixtures, indicated minimal urinary α-amino nitrogen excretion from L-amino acid diets but considerable amounts when the D-Lisomers were fed. The ammonia nitrogen excretion also depended on the amino acid complement of the diets, and was minimal on the first diet (Diet 26). Faecal nitrogen excretion was small and constant indicating an essentially quantitative absorption (Birnbaum et al, 1957 a). Further experiments on the non-essential nitrogen component of the diet were conducted by adding various amino acids and nitrogenous compounds to a basal diet containing 9.5g of essential amino acid nitrogen, at the expense of glucose, to a level of 25g nitrogen/Kg of diet. L-alanine, ammonium L-glutamate, L-glutamine, ammonium

aspartate and L-proline as the sole component were all found to be good sources of non-essential nitrogen for promoting growth. D-alanine and D-arginine both produced some acceleration of growth, and ammonium acetate was better than urea or glycine (Birnbaum et al, 1957 b).

The effect of the nature of the carbohydrate source on rat-growth was investigated and a sucrose based diet was found to give marginally the best growth response with D-glucose, invert sugar and D-fructose giving a decreasing order of growth response and intake. D-glucosamine based diets caused a marked reduction of dietary intake and weight gain (Winitz et al, 1957 a). The role of arginine on growth was also investigated by feeding diets of varying non-essential nitrogen composition with and without L- or D-arginine added. Without arginine the three diets were poor but equally efficacious, but the addition of D- or L-arginine enhanced growth with the diet containing all the non-essential amino acids to a greater extent than with the other diets (Winitz et al, 1957 b).

The final report in the series described the formulation of the complete, nutritionally adequate, chemically defined, liquid diet composed of eighteen crystalline L-amino acids, water soluble vitamins, pertinent salts, glucose, fat soluble vitamins and ethyl linoleate. The use of Tween 80 gave a stable emulsion from which no separation of the fat components occurred. Weanling rats fed this formulation ad-libitum for a period of three months showed no signs of fatty acid deficiency and grew at the same rate as controls that were provided with the fat soluble vitamins in corn oil as a supplement (Greenstein

et al, 1960). Thus Abderhaldens statement in 1912 that a synthetic diet could be produced was eventually achieved with a chemically defined diet in 1960.

Formulation problems with later amino acid based diets were encountered by some workers. Levenson et al (1971) found that rats developed haemolytic anaemia and pancreatic acinar atrophy when fed their liquid elemental diet formula, and suggested that cysteine ethyl ester hydrochloride and menadione were interacting in their particular formulation. Basic animal studies at Johnson and Johnson during the development of their amino acid based diet, Jejunal, revealed a deficit of choline in the formulation, which produced fatty livers, but was easily corrected. (Campbell et al, 1973).

Human Studies with Amino Acid Based Diets

Having completed their animal studies and developed a complete elemental diet, the Greenstein group extended their studies to humans. Clinical trials of their diet in patients with slowly progressing neoplasms indicated that the diet maintained body weight and normal organ function with the establishment of a positive nitrogen balance in adult man (Cough et al, 1960). Their studies were then extended to long term feeding in normal healthy volunteers and the carbohydrate nature of the diet was shown to influence serum cholesterol levels (Winitz et al, 1964). Further impetus to the studies came from the aero-space programme and the evaluation of chemical diets continued as nutrition for man in space (Winitz et al, 1965).

The studies on human volunteers were reported fully in 1970 after extended feeding of 24 subjects for 22 weeks (Winitz et al, 1970 a). The original formulation included cysteine ethyl ester-HCL and menadione but these were later replaced by methionine and safflower oil. Other variations in the diets included replacement of 25% of the glucose by sucrose, and tyrosine by phenylalanine. All subjects remained in a satisfactory physical state and no abnormalities were found in blood chemistry, haematology or urine analysis. A marked reduction in faecal bulk and frequency of defaecation was noted. Diets containing glucose as the sole carbohydrate source reduced serum cholesterol within four weeks and adding 25% sucrose reversed this change, again after three weeks. The ratio of free to esterified cholesterol remained essentially constant.

A second report of the studies examined the effects of the diets on gut microflora populations (Winitz et al, 1970 b). The glucose based diet led to a marked reduction of the faecal microbial population but the sucrose based diet resulted in a different alteration, with certain bacterial types, including bacteriodes, showing a greater propensity to survive than others. These findings were later disputed and the techniques criticised by other workers, who could not demonstrate the same reduction of the faecal microflora, but the carbohydrate was not pure glucose in the commercial elemental diets used (Crowther et al, 1973; Bounous and Devroede, 1974).

Schwarz Bio-Research produced and marketed an amino acid based elemental diet known as Codelid, and this diet was used by Thompson, Stephens and Randall for the management of a patient with extreme short bowel syndrome (Thompson et al, 1969). These workers went on to demonstrate the usefulness of the diet in the management of various catabolic states including short bowel syndrome, pancreatic insufficiency and ulcerative colitis, with the attainment of a positive nitrogen balance (Stephens and Randall, 1969). The mortality rate in patients with gastro-intestinal fistulas was reduced from the 40-70% generally observed to 15.4% in 13 patients treated with the elemental diet by the same group (Bury et al, 1971).

Jejunal, another amino acid based diet which was marketed in the States, was found to be useful in patients undergoing preparation for abdominal surgery and patients with feeding problems. Minimal variation in blood coagulation and chemistry indicated that the diet was safe as used in the patients studied (Miller and Taboada, 1975).

Diets Based on Protein Hydrolysates

In 1967 Bounous and co-workers demonstrated that a diet formulated from a commercially available casein hydrolysate, plus sucrose and fat emulsion, prevented the denudation of intestinal villi produced by hypovolemic shock in dogs, when fed for the preceeding three days (Bounous et al, 1967 a). In a further report they coined the term 'elemental' for their diet formulation and showed an increased rate of incorporation of labelled adenine into ATP of the intestinal mucosa after a 3 day period of feeding the diet in dogs

(Bounous et al, 1967 b). The elemental diet was also shown to be beneficial in the management of the intestinal lesion produced by 5-fluorouracil in the rat (Bounous et al, 1971 a) and man (Bounous et al, 1971 b).

The commercial diet Flexical, developed by Mead Johnson, was based on the formulation of these workers and, unlike the amino acid based diets, it contains an appreciable amount of fat. The protein hydrolysate contains approximately 70% free amino acids and 30% small peptides. The product initially released for testing in 1969 was designated Mead Johnson 3200-AS and the effects of this diet were compared with a control non-hydrolysed liquid diet (3200-AU) in normal volunteers (Perrault et al, 1973). Neither diet had much effect on serum biochemical and haematological factors over the 12 day period and it was concluded that it was safe for human use, for this length of time. Transit time of radio-opaque markers was significantly increased by both liquid diets and serum cholesterol reduced. Trypsin and bile acid secretion, measured by duodenal perfusion, were somewhat reduced by the elemental diet.

Bounous and co-workers then reported their experience with the use of Flexical in 22 patients. It was found to be useful in those with intestinal disorders, including 9 with Crohn's disease, 3 with fistula of the alimentary tract and 2 with short bowel syndrome. The diet was also used successfully for preparation of the bowel for surgery and in the management of severe acute trauma (Bounous et al, 1974). Similar uncontrolled retrospective studies of the use of Flexical in the management of intestinal disorders were

reported by Voitk and his co-workers in 1973. They initially reported on 13 patients with inflammatory bowel disease including patients with Crohn's disease, ulcerative colitis and radiation enteritis, some of whom had short gut syndrome or a fistula. In all but one patient a positive nitrogen balance and weight gain was achieved (Voitk et al, 1973 a). Treatment of 29 patients with 38 fistulas resulted in spontaneous closure of 75% of the fistulas, while a positive nitrogen balance was achieved, indicating that Flexical supplied nutritional needs without aggravating the fistula by corrosive faecal drainage (Voitk et al, 1973 b). Flexical was also used during the adaptive phase of short gut syndrome in eight patients and successful adaptation was achieved in four patients, who were then able to tolerate normal food (Voitk et al, 1973 c).

Comparison of Commercially Available Elemental Diets

Only two elemental diets have been marketed in Britain, namely Vivonex (Eaton Laboratories Ltd.) and Flexical (Mead-Johnson). In addition, Aminutrin and Calonutrin (Geistlich) have been marketed together as an elemental diet, but do not provide a satisfactory balanced preparation as they contain no fat and cannot be considered as a nutritionally complete diet. Vivonex was the first to become available in March 1974, and so studies on elemental diets concentrated on Vivonex, before Flexical appeared in the middle of 1975. Other amino acid based diets, Codelid, Jejunal and Precision LR, were also marketed in America. While the work reported in this thesis was in progress, a case was reported comparing the absorption of different

elemental diets in a patient with short bowel syndrome, following extensive small intestinal resection and total colectomy for Crohn's disease. The patient was fed by nasogastric tube and absorption was studied by comparing the volume fed with volume of jejunostomy output and measuring fat, nitrogen and phosphorus concentrations. Nitrogen retention was greatest with Vivonex HN and Flexical, but all the diets except Flexical produced profuse drainage from the jejunostomy. This was attributed to the higher fat content of Flexical (Simko and Linsheer, 1976).

The composition of Vivonex and Flexical is shown in Tables 2.1 and 2.2. The main differences are in the fat content and the nature of the nitrogen source. Vivonex is virtually fat free but Flexical supplies 30% of calories as fat. Vivonex contains synthetic amino acids, whereas Flexical is based on enzymatically hydrolysed casein and contains 30% of the amino acids as oligopeptides.

Total α-amino nitrogen absorption by isolated loops of rat intestine was greater from pancreatic hydrolysates of protein than the corresponding mixtures of free amino acids (Crampton et al, 1971). Later studies in man demonstrated that total amino acid absorption was greater from a tryptic hydrolysate consisting of oligopeptides and amino acids than the corresponding free amino acid mixture. The absorption of individual amino acids was measured and some were found to be better absorbed from the free amino acid mixture and others from the hydrolysate (Silk et al, 1973). Thus in normal rats and man peptides present a more rapidly

absorbed nitrogen source than free amino acids, but this may not necessarily be true in certain disease states.

The clinical findings with Flexical in patients with inflammatory bowel disease, short gut syndrome and fistulas of the alimentary tract (Voitk et al, 1973 a,b,c; Bounous et al, 1974) are comparable to those reported with amino acid based diets (Thompson et al, 1969; Stephens and Randall, 1969; Bury et al, 1971) and so it appears that complete hydrolysis of protein to free amino acids confers no real advantage for the clinical applications of elemental diets.

Protein	Carbohydrate	Fat
Pure L-amino acids 33.04g/day	Glucose solids 414g/day	Purified safflower oil 2.61g/day
	g/day		g/day	
Isoleucine	1.65	Monosaccharides	4.14	Triglyceride of Linoleic acid 2.09 g
Leucine	2.61	Disaccharides	24.34	
Lysine	1.96	Trisaccharides	33.12	
Methionine	1.69	Tetrasaccharides	28.98	
Phenylalanine	1.87	Pentrasaccharides	322.92	
Threonine	1.65			
Tryptophan	0.51			
Valine	1.81			
Alanine	1.75			
Arginine	3.22			
Aspartic acid	3.75			
Glutamine	6.19			
Glycine	2.86			
Histidine	0.79			
Proline	2.35			
Serine	1.21			
Tyrosine	2.05			

TABLE 2:1 COMPOSITION OF THE ELEMENTAL DIET VIVONEX.

The recommended daily intake of 6 x 80g packets supplies 1800 calories. Nitrogen, carbohydrate and fat are supplied as shown in the table, together with vitamins and minerals.

Protein		Carbohydrate		Fat	
Enzymatically hydrolysed casein (plus added amino acids *)		Corn syrup solids, Sucrose, tapioca starch, citric acid.		Soy oil, fractionated coconut oil	
45.0g/day		308g/day		63.0g/day	
	g/day		g/day	Fatty acids	g/day
Isoleucine	2.35	Sucrose	201.8	Octanoic	10.9
Leucine	4.82	Dextrin	96.8	Decanoic	3.9
Lysine	4.03	Citrate	9.4	Palmitic	6.2
*Methionine	2.12			Stearic	2.1
Phenylalanine	2.24			Oleic	21.7
Threonine	2.19			Linoleic	13.0
*Tryptohan	0.71			Linolenic	4.8
Valine	3.54				
Alanine	1.69				
Arginine	1.87				
Aspartic acid	3.79				
Glutamic acid	10.97				
Glycine	1.12				
Histidine	1.41				
Proline	5.11				
Serine	2.98				
*Tyrosine	1.18				

TABLE 2:2 COMPOSITION OF THE ELEMENTAL DIET ELEMICAL

The recommended daily intake of 454g supplies 2000 calories. Nitrogen, carbohydrate and fat are supplied as shown in the table together with vitamins and minerals.

CHAPTER 3

BILE ACID METABOLISM

Structure and Terminology

The common mammalian bile acids are hydroxyl-substituted derivatives of 5β-cholanoic acid (cholanic acid), the structure of which is shown in Figure 3.1. The substitution of the individual bile acids important in man and the rat, together with their trivial names and abbreviations used, are shown in Table 3.1. The two primary bile acids synthesised from cholesterol in the liver are cholic and chenodeoxycholic acid. Deoxycholic and lithocholic acid are secondary bile acids which result from 7α dehydroxylation by anaerobic intestinal bacteria (Mosbach, 1972).

Under normal physiological conditions free bile acids do not occur in bile, and the bile acids are secreted as the taurine or glycine conjugates with a peptide link to the carboxyl group of the side chain (Schersten, 1967).

Synthesis

Cholic and chenodeoxycholic acids are synthesised in the mammalian liver from cholesterol. The transformation involves the following changes to the sterol molecule:-

a. Stereospecific reduction of the double bond between C5 and C6 to yield the 5β-cholestane configuration.
b. Epimerization of the 3β hydroxyl group.
c. Removal of the terminal three carbons of the side chain leaving a carboxyl function at C24.
d. Introduction of hydroxyl groups C7 and C12.

In the main synthetic pathway transformations of the nucleus precede degredation of the side chain, but alternative pathways for chenodeoxycholic synthesis have been proposed.

The CoA esters of bile acids are conjugated by a lysosomal acyl transferase (Mosbach, 1972).

The first committed step of bile acid synthesis is the introduction of a 7α hydroxyl group into the cholesterol molecule and is catalysed by cholesterol 7α hydroxylase which is a microsomal mixed-function oxidase requiring cytochrome P450, NADPH and oxygen. Free, rather than esterified, cholesterol is the substrate and the enzyme is rate limiting in bile acid biosynthesis in that there are no rate-limiting steps beyond this in the pathway.

Interruption of the enterohepatic circulation of bile acids, by creating a bile fistula or by cholestyramine treatment, causes a marked rise in bile acid synthesis and cholesterol 7α hydroxylase activity increases in parallel. Since there is a delay of 24 hours and the rise is prevented by actinonycin D it is probably due to induction of some component of the enzyme system rather than activation of pre-existing enzyme. The activity of HMGCoA reductase, which is the rate limiting enzyme of cholesterol synthesis, also increases (Myant and Mitropoulous, 1977).

Bile acid synthesis is apparently under negative feedback control and varies inversely with hepatic bile acid flux (Shefer et al, 1969; Low-Beer et al, 1972; Mosbach, 1972).

Enterohepatic Circulation.

Bile acids are conserved by undergoing enterohepatic circulation: they are secreted by the liver and pass into the intestine where they are absorbed in large part by the ileum and return to the liver by way of the portal circulation.

Bile acids returning to the liver in the portal circulation are effeciently taken up by a carrier mediated process, although the process is less effecient for unconjugated than conjugated bile acids (O'Maille et al, 1967). In the liver any free bile acids are reconjugated with glycine or taurine in the same manner as newly synthesised bile acids, and then secreted into the bile canaliculi by a specific active transport process which has a capacity far exceeding the usual demands imposed on it (Wheeler, 1972).

Bile acids associate with phospholipid and cholesterol to form mixed micelles and the bile may then be stored in the gallbladder during fasting or pass directly into the duodenum during digestion.

In the upper small intestine bile acids participate in the digestion and absorption of fats including triglycerides, cholesterol and the fat soluble vitamins. They aid the emulsification of ingested fat, thus enhancing lipolysis, and solubilize the fatty acids and β-monoglycerides produced in micelles (Holt, 1972). In this way most of the lipids are absorbed in the jejunum, but most of the bile salts remain in the intestinal content (Borgstrom et al, 1957).

Under normal circumstances bile acids are mainly absorbed in the ileum of rats (Baker and Searle, 1960) and man (Borgstrom et al, 1963) by an active transport mechanism (Lack and Weiner, 1961). Dihydroxy bile acids are absorbed less efficiently than cholic acid, and taurine conjugates are transported more rapidly than the glycine conjugates

(Lack and Weiner, 1966). Small quantities of bile acids, especially any unconjugated bile acids and the glycine conjugates of dihydroxy bile acids, are absorbed by passive non-ionic diffusion along the length of the intestine (Dietschy, 1968).

Bacterial modification of bile acids is confined mainly to the colon and terminal ileum under normal circumstances (Gorbach et al, 1967). The principle modifications are deconjugation and 7α dehydroxylation, with further changes including oxidation of hydroxyl groups to keto groups and epimerization of hydroxyl groups (Dowling, 1972). The deconjugating enzyme is widely distributed among anaerobic intestinal bacteria such as Bacteroides, Veillonella, Bifido bacteria and some strains of Clostridia (Hill and Drasar, 1968).

The modification of bile acids by bacteria was reviewed in 1972 by Lewis and Gorbach. Deoxycholic acid formed in the terminal ileum and colon is reabsorbed, probably mostly in the colon, and its glycine and taurine conjugates normally constitute about 22% of total bile acids in human bile (Sjövall, 1960). Little of the lithocholic acid formed is absorbed, since it is insoluble at the pH of the distal intestine.

Intestinal absorption of bile acids thus depends on the nature of the individual bile acids, the extent of bacterial deconjugation and modification, and is also influenced by the rate of intestinal transit (Meihoff and Kern, 1968) and the amount and nature of unabsorbed dietary

residue which may bind bile acids (Eastwood and Boyd, 1967). Unabsorbed bile acids are further modified by bacteria and excreted in the faeces.

Dynamics of the Enterohepatic Circulation

The pool size of bile acids, that is the total amount in the body, is normally constant and faecal loss is balanced by synthesis of new bile acid in the liver. In man there is normally a 3-5g pool which circulates about 6 to 10 times per day, with a daily loss in the faeces of 200-600mg (Dowling, 1972). The pool size of a given bile acid can best be determined from the slope of the specific activity decay curve measured over a five day period on duodenal bile samples, following injection of radiolabelled bile acid (Hofmann and Hoffman, 1974). This method also determines the biological half-life of the bile acid.

Experiments in the Rhesus monkey with controlled interuption of the enterohepatic circulation have shown that 20% interuption can be compensated for by a tenfold increase in hepatic synthesis, but that greater interuption results in a marked reduction in bile acid secretion (Dowling et al, 1970).

Resection of the terminal ileum, or damage to it in Crohn's disease, results in malabsorption of bile acids with a greatly increased turnover of bile acids (Meihoff and Kern, 1968). This can be compensated for to a certain extent by a large increase in hepatic synthesis, and steatorrhoea is mild in patients with resection of less than 100cm. With larger resections the liver cannot keep pace with the increased faecal loss and steatorrhoea may be

severe (Hofmann, 1972).

The total bile acid pool was found to be enlarged in patients with coeliac disease, with an increase in taurocholic acid half life but a normal synthetic rate. There was a strong correlation between half-life and pool size and it was suggested that cholecystokinin release from the damaged upper small intestine was impaired and gallbladder contraction decreased. This resulted in a very sluggish entero-hepatic circulation of bile acids with reduced hepatic surveillance and derepression of bile acid synthesis (Low-Beer et al, 1973).

The Role of Bile Acids in Lipid Absorption

Bile acids and their conjugates are aromatic detergents. A detergent is a soluble amphiphile in which the polar group is strong enough to overcome the non-polar group and confer solubility in water. Above a certain concentration, the critical micellar concentration (CMC), polymolecular aggregates or micelles are formed with the polar groups facing into water and protecting the non-polar groups inside the micelle. The weakly hydrophilic hydroxyl groups are all on one side of the common bile acid molecules so that they have a non-polar 'back,' a polar 'front' and a strongly hydrophilic carboxyl or sulphate group. Pure bile acid micelles are not efficient solubilizers of lipids or emulsifiers, but become very efficient when expanded by other polar lipids, such as lecithin, to form mixed micelles.

All lipids of physiological interest are to some extent polar and the insoluble ones, which require micellar

solubilization to be incorporated into the aqueous phase, are divided into two classes

(i) insoluble, non-swelling amphiphiles, which includes di- and triglycerides, cholesterol and fat soluble vitamins.

(ii) insoluble swelling amphiphiles, which includes phospholipids, monoglycerides and some ionised long chain fatty acids. These have a polar group which is strong enough to allow a certain interaction with water.

The latter align themselves with bile acids to expand the micelle and the resulting mixed micelle has a much greater ability to solubilize non-swelling amphiphiles such as cholesterol (Carey and Small, 1972). The schematic structure of these micelles is illustrated in Figure 3.2.

The evidence for the existence of micelles in vivo includes ultra-centrifugation of aspirated intestinal contents which reveals a water clear phase rich in bile salts, monoglycerides and fatty acids (Hofmann and Borgstrom, 1964).

In the absence of bile, triglyceride absorption is only reduced to 50-75% but fatty acids appear free in portal blood rather than as triglycerides incorporated into chylomicrons in thoracic duct lymph (Borgstrom, 1953). Whether this reflects a direct effect of bile acids on mucosal re-esterification is uncertain (Holt, 1972).

Although not essential for the absorption of triglycerides, bile acids appear to enhance the overall process of lipolysis in vitro and in vivo. The main effects

are probably

(i) aiding emulsification of fat by mixed micelles and thus increasing the surface area. Lipolysis of long chain triglycerides occurs only at the oil-water interphase and releases free fatty acids plus β-monoglycerides (Sarda and Desnuelle, 1958).

(ii) Solubilising the products of lipolysis in a micellar solution thus enhancing transport across the unstirred layer and into the microvilli. This seems to be rate-limiting, rather than the rate of lipolysis, in the absence of bile (Holt, 1972).

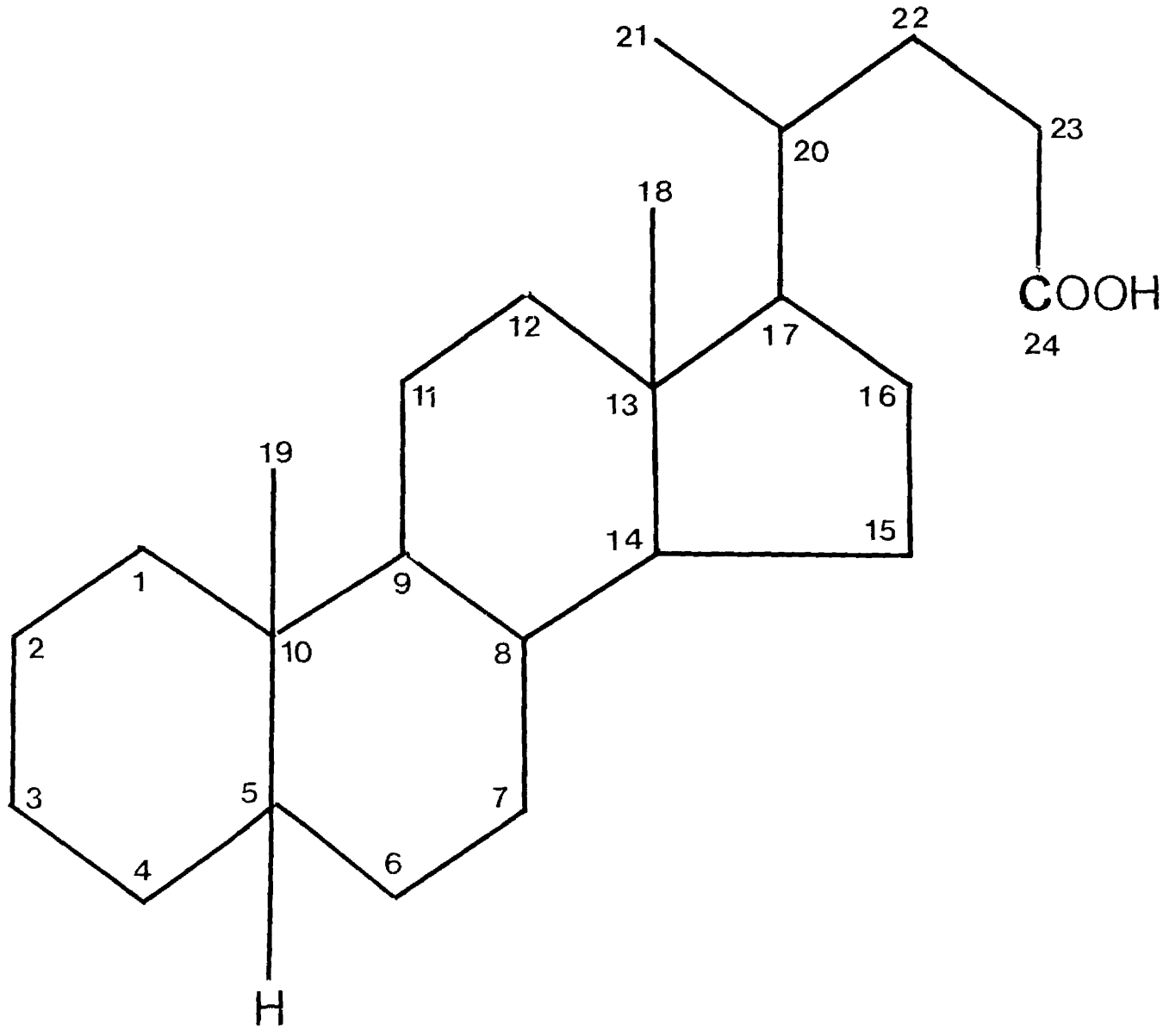
Bile acids have been shown to be essential for the absorption of fat soluble vitamins and cholesterol, which require solubilization in mixed micelles for transport across the unstirred water layer to be absorbed at the microvilli of the mucosal cells (Siperstein et al, 1952; Holt, 1972). Cholesterol is never completely absorbed since the monoglycerides, phospholipids and fatty acids necessary for the formation of the mixed micelles are absorbed more rapidly than the cholesterol (Simmonds et al, 1967).

Differences in Bile Acid Metabolism Between the Rat and Man

One obvious difference between the rat and man which is concerned with bile acid metabolism is the absence of a gallbladder in the former. Throughout the day the bulk of the bile acid pool is contained in the small intestine and caecum of the rat (Fo, 1976), whereas in man bile is stored in the gallbladder between meals (Wheeler, 1971). Cholecystectomy reduces the bile acid pool size and the bile

contains more deoxycholate (Pomare and Heaton, 1973). Another difference between the two species is the presence in the rat liver of enzymes capable of hydroxylating bile acids at the 7α and 6β positions. Thus, deoxycholic acid formed in the gut can be rehydroxylated to cholic acid in the rat (Bergstrom et al, 1960; Thomas et al, 1964). Hydroxylation at the 6β position gives rise to bile acids of the muricholate series plus hyodeoxycholic and hyocholic acids. Hyodeoxycholic acid arises by a combination of 6β hydroxylation in the rat liver and the action of gut bacteria, which epimerise the 6β hydroxyl group to the α position, probably via the keto derivative (Einarsson, 1966).

FIGURE 3.1. STRUCTURE OF 5B CHOLANOIC ACID (CHOLANIC ACID) SHOWING NUMBERING OF THE CARBON ATOMS


21
22
23
20
18
COOH
24
12
17
11
13
16
19
14
15
1
9
2
10
8
3
5
7
4
6
H

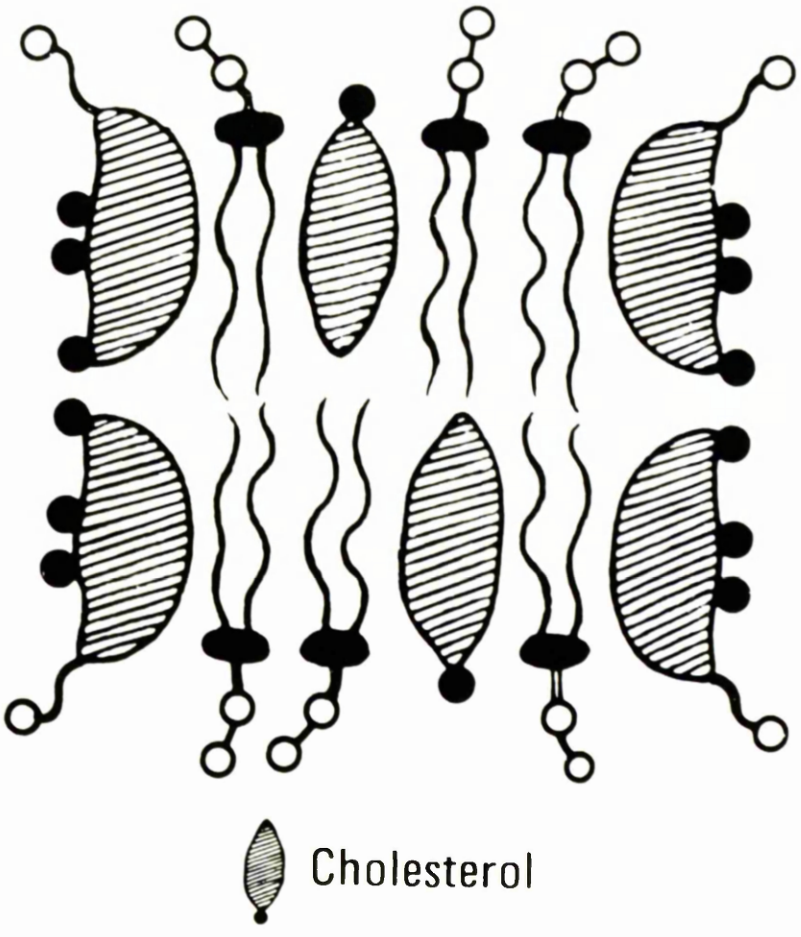


FIGURE 3.2. SCHEMATIC STRUCTURE OF A MIXED MICELLE OF BILE ACID AND PHOSPHOLIPID CONTAINING SOLUBILIZED CHOLESTEROL

Hydroxyl-substitution of cholanic acid	Trivial name	Abbreviation
3α, 7α, 12α	Cholic acid	C
3α 7α	Chenodeoxycholic acid	CDC
3α 12α	Deoxycholic acid	DC
3α	Lithocholic acid	LC
3α 7β	Ursodeoxycholic acid	UDC
3α 6α	Hyodeoxycholic acid	HDC
3α 6β 7α	α- muricholic acid	α-MC
3α 6β 7β	β - muricholic acid	β -MC
3α 6α 7β	ω - muricholic acid	ω -MC

TABLE 3:1 STRUCTURE OF INDIVIDUAL BILE ACIDS

The common mammalian bile acids are hydroxyl-substituted derivatives of 5 B-cholanoic acid (cholanic acid).

CHAPTER 4

STRUCTURE AND FUNCTION OF THE SMALL INTESTINE

Dynamics of the Epithelium

The mucosal lining of the intestinal tract is in a constant state of renewal and the enteric epithelium has the fastest rate of turnover for any tissue in the body under normal circumstances. The enteric epithelium is completely replaced within two to three days in mice and rats (Leblond and Walker, 1956; Abrams et al, 1963) and man (Lipkin et al, 1963; MacDonald et al, 1964).

A diagramatic representation of the kinetic compartments in the crypts and villi of small bowel mucosa is shown in Figure 4.1. Much of the knowledge of the compartmentalization of the cell renewal system is derived from experiments in rodents, but it is virtually certain that a similar arrangement obtains in man (Watson and Wright, 1974). The enterocytes are regular close packed columnar epithelial cells with a distinct brush border of microvilli each about 1μ in length and 0.1-0.2μ in width. The only break in the enterocyte layer is at the villus tip where extrusion zones show the site of desquamation (Creamer, 1974).

Columnar (absorptive) cells outnumber the other three major cell types, which are mucous (goblet) cells, enteroendocrine (argentaffin) cells and Paneth cells. All four cell types are thought to arise from the same precursor, the undifferentiated crypt base columnar cell (Cheng and Leblond, 1974).

Cell division in the enteric epithelium is restricted to the base of the crypts, where cells have high activities

of thymidine kinase and other enzymes involved in nucleic acid synthesis (Imondi et al, 1969). The M phase of actual mitosis occupies only a brief part (one hour) of the complete cell cycle which lasts for 10-17 hours in rodents and at least 24 hours in man. DNA synthesis is confined to a discrete period (S phase), but RNA and protein are actively elaborated throughout the S phase and during the premitotic (G_2) and postmitotic (G_1) gaps (Eastwood, 1977). The rate of turnover is so rapid that cells do not normally enter the prolonged interphase (G_o) found in other more slowly renewing tissues.

After at least two divisions within the crypt, cells migrate onto the villus, having lost the ability to incorporate thymidine and undergo further mitosis (Lipkin, 1973; Eastwood, 1977). Entry from the proliferative compartment to the maturation compartment is accompanied by differentiation into the mature columnar cell containing enzymes associated with its primary function of nutrient absorption (Imondi et al, 1969; Lipkin, 1973). Cell migration is completed more rapidly in the ileum, rather than in the jejunum, as a result of the decreasing aboral gradient of villus height along the small bowel (Altmann and Ehesco, 1967).

Under normal steady rate conditions cell loss from the villus tip is balanced by cell birth at the crypt base. The number of villus cells regulates crypt cell proliferation in the epithelium of the small intestine by a feedback control mechanism (Rijke et al, 1976). Crypt cell proliferation is affected by physiologic variables including age, diurnal rhythm and oestrus cycle (Leblond and Walker, 1956).

In coeliac disease the increased rate of cell loss with villus atrophy, resulting from gluten damage, is accompanied by a marked increase in crypt cell production. The proliferative compartment in the crypts increases and the cell cycle time is halved with a resulting six-fold increase in cell production from the crypts (Watson and Wright, 1974).

In germ free animals villus transit time is increased and cell turnover is reduced so that the ratio of villus height to crypt height is increased (Abrams et al, 1963; Galjaard et al, 1972).

Absorption of Water and Electrolytes

Water intake is comparatively small (1½-2l) compared with the total fluid load presented to the small intestine. Salivary, gastric, biliary, pancreatic and mucosal secretions total about 7l per day in man. All the evidence suggests that water absorption is passive and accompanies net movement of solute out of the lumen in isotonic proportions.

The duodenum is freely permeable to fluid and electrolytes, and following the ingestion of a meal gastric contents are rendered isotonic by the time the upper jejunum is reached (Fordtran and Locklear, 1966). The intubation studies of Borgstrom and co-workers indicated that the bulk absorption of protein, starch and fluid was performed by the proximal half (100 cm) of the small intestine (Borgstrom et al, 1957). The jejunum, therefore, absorbs large quantities of water, sodium and chloride from a solution which is isotonic. Perfusion of the jejunum with saline results in very little demonstrable absorption but glucose, at concentrations greater

than 14nM, promotes absorption of sodium, chloride and water (Sladen and Dawson, 1969). Glycine, leucine and galactose all have a similar effect, and the effects may be additive (Malin et al, 1970). It seems likely that glucose promotes net sodium transport in a direct fashion and that water transport is a secondary consequence of this. Crane's hypothesis, relating glucose and sodium transport, suggests that these two solutes combine with a common membrane carrier which facilitates entry of both into the mucosal cell (Crane, 1965). The driving force for sodium transport comes from the sodium pump situated at the basal membrane of the mucosal cells. These membrane bound ATPases (Skou, 1965) maintain the low intracellular sodium concentration by actively transporting sodium out of the cells. Chloride appears to accompany sodium by passive diffusion.

In contrast to the jejunum, sodium absorption in the ileum can occur independently of other solutes and take place against a large concentration gradient (Fordtran et al, 1968).

Carbohydrate Digestion and Absorption

Most of the carbohydrate ingested is in the form of starch, sucrose and lactose. The α-amylases of saliva and pancreatic juice are both capable of hydrolysing the amylose (α 1;4 links) and amylopectin (branched chains with α1;6 links as well) constituents of starch, but it is probable that the major part of their digestion is carried out in the small intestine by the pancreatic enzyme (Dahlquist and Borgstrom, 1961; Forel and Gray, 1970). The α 1-6 link is not hydrolysed and so the products of digestion in the

small intestinallumen are maltose, maltotriose and α-limit dextrins with very little glucose being formed (Roberts and Whelan, 1960), together with unchanged sucrose and lactose.

For many years it was considered that the luminal phase of carbohydrate digestion, that is the hydrolysis of disaccharides, took place by the action of enzymes secreted into the lumen in the 'succus entericus'. Support for the concept of surface or membrane digestion was provided by the localization of the disaccharidases to the terminal membrane of the brush border (Nicholz and Crane, 1965) and the demonstration of enzyme rich knobs on the surface of the microvillus membrane (Oda and Seki, 1965). The brush border contains several disaccharidases including lactase, sucrase, maltase and the so-called 'iso-maltase'. The latter acts on the α 1:6 link of the α-limit dextrins, and the oligosaccharides produced are further hydrolysed to glucose by the action of pancreatic α-amylase and the brush border enzymes (Alpers and Solin, 1970). Glucose and galactose are absorbed by the same active carrier process, which is located in close proximity to the disaccharidases in the brush border membrane. The energy for transport is provided in part by the sodium concentration gradient which is maintained by the sodium pump. The sodium dependance of glucose absorption in vivo has not been clearly demonstrated in man, although perfusion studies have shown a small reduction in glucose absorption against a concentration gradient when sodium was replaced with mannitol (Olsen and Ingelfinger, 1963).

Protein Digestion and Absorption

Protein digestion is initiated in the stomach by gastric proteinases (pepsins) and continued in the small intestine by the pancreatic enzymes including trypsin, chymotrypsin, elastase and carboxypeptidases, which are secreted as inactive precursors and activated by the small intestinal brush border enterokinase. Borgstrom et al found that only 10-15% of dietary protein was hydrolysed in the stomach but that 50-60% was rapidly hydrolysed when the duodenum was reached, and that most of the protein was absorbed in the proximal 50-60cm of the jejunum (Borgstrom et al, 1957). The products of hydrolysis are amino acids and small peptides.

In 1953 Wiseman demonstrated that amino acids were actively transported (Wiseman, 1953) and there are several different transport systems. The following transport groups have been suggested (Matthews and Laster, 1965).

(i) monoamino-monocarboxylic acids

(ii) dibasic amino acids

(iii) N-substituted amino acids

(iv) glycine

The possibility that protein was transported as peptide and hydrolysed intracellularly was seriously investigated after Newey and Smyth demonstrated that intraluminal hydrolysis followed by mucosal uptake could not account for the absorption of amino acids from dipeptides (Newey and Smyth, 1962). A transport system for peptides separate from that for amino acids and also more efficient, with the apparent absorption of intact peptides, was demonstrated in

rats and man (Matthews et al, 1969; Adibi, 1971). Perfusion studies in man demonstrated that total amino acid absorption was greater from a tryptic hydrolysate containing oligopeptides and amino acids than from the corresponding free amino acid mixture. Some of the individual amino acids were better absorbed from the free amino acid mixture and some from the hydrolysate (Silk et al, 1973).

Two mechanisms for peptide hydrolysis and transport independant of the usual free amino acid carriers have been proposed. The first involves the uptake of peptide by a special carrier with hydrolysis by a cytoplasmic peptidase, and the second proposes that peptides are hydrolysed at the membrane and taken up by a special carrier mechanism for amino acids liberated by the action of brush border peptidase (Kim, 1977).

Peptidase activities are high in intestinal mucosal cells but only a trace of such activity is found in luminal contents. The peptidase activity of the mucosal cells is localized in two subcellular fractions, the brush border and cytoplasmic fractions. The brush border fraction has less than 12% of the cellular activity toward dipeptide substrate but as much as 60% of that toward tripeptides (Kim et al, 1974).

The mechanisms and importance of peptide absorption are still being investigated. In patients with coeliac disease the rate of jejunal absorption of amino acids is markedly reduced whereas that of dipeptides is less affected (Adibi et al, 1974).

Fat Digestion and Absorption

The role of bile acids in fat digestion and absorption has been discussed in Chapter 3.

Once the B-monoglyceride and fatty acid, which are the products of triglyceride digestion, have entered the mucosal cell they are mainly reconverted into triglyceride (Dawson, 1967). This takes place either by the ∝-glycerophophate pathway or alternatively by acylation of the monoglyceride with CoA derivatives of the fatty acids. It is thought that about 60% of the monoglyceride is preserved by this latter pathway, but the relative utilization of the two pathways depends on the availability of the various substrates (Senior and Isselbacher, 1963).

Triglycerides and cholesterol are transported out of the cell as chylomicrons or very low density lipoproteins (VLDL) into the lymphatics. Chylomicrons contain 81-97% triglyceride, 2-9% phopholipid, 3% cholesterol and 2% protein. Unsaturated fatty acids are transported only in the chylomicron fraction while saturated fatty acids are transported by VLDL as well as chylomicrons (Ockner et al, 1969).

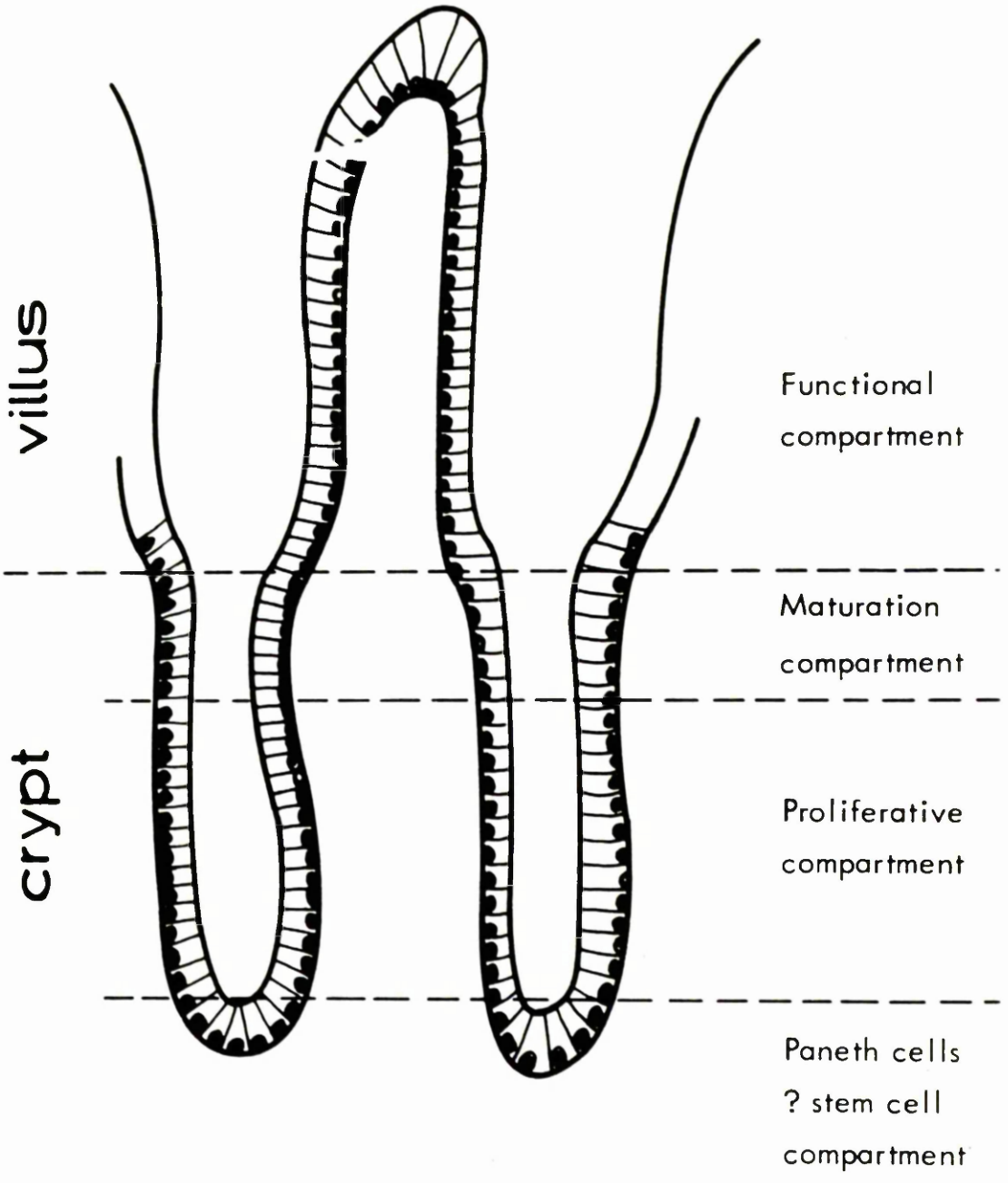


FIGURE 4.1 DIAGRAMATIC REPRESENTATION OF THE KINETIC COMPARTMENTS OF THE CRYPTS AND VILLI OF SMALL BOWEL MUCOSA

CHAPTER 5

MEASUREMENT OF FAECAL BILE ACID EXCRETION IN MAN AND THE RAT

INTRODUCTION

Two major gas-liquid chromatographic methods for measurement of faecal bile acids were published in the late 1960's.

The method of Grundy et al (1965) involves a thin-layer chromatographic purification step in addition to solvent extractions, and for samples containing less than 200µg of bile acids per g of faecal homogenate aflorisil column step was also included. Bile acids were chromatographed as the trimethylsilyl ethers of the methylesters, and the integrated peak area following the 5α-cholestane standard peak was calculated to give total faecal bile acids. Radioactively labelled deoxycholic acid was used as a recovery marker for the steps preceeding GLC analysis.

In 1968 Evrard and Janssen published a simpler, shorter method employing 23-Nordeoxycholic acid as an internal standard to quantitate recovery at the various extraction and purification steps and also for the GLC stage. The original method chromatographed the bile acids as the ketonic methyl esters on 1% JXR columns and didn't separate deoxycholic and chenodeoxycholic acids. Modification of the chromatographic conditions employing a phenyl-methyl silicone phase (OV-17, 50 % phenyl) and omitting the oxidation step, as reported by Allan et al (1974), allows separation of deoxycholic and chenodeoxycholic acids.

This modified Evrard and Janssen method was employed in the investigation of the effect of elemental diets on faecal bile acid excretion reported in the following chapters.

OUTLINE OF METHOD

Preparation of Samples

For human studies a faecal collection was made over a 5 day period and homogenised using a Silverson emulsifier with a minimum volume of water.

The total faecal weight and weight of the homogenate were noted. A weighed aliquot (50-100g) was then freeze-dried, reweighed and a portion (0.3g) taken for analysis.

Rat faeces were normally pooled in seven day samples, dried and finely ground in a pestle and mortar or using an electric mill. For analysis an aliquot (1g) of the powder was used.

Extraction of Bile Acids

Exactly 5 ml of glacial acetic acid containing the internal standard, 23-nordeoxycholic acid (300µg/ml) was added to the weighed, powdered faeces in an 30ml quickfit tube, and the bile acids extracted by heating for 1 hour at 120°C in a Statim dry block. The samples were then cooled, 6ml of toluene added and mixed well. Approximately 3 ml of the supernatant was transferred to a second tube and the solvents evaporated in a water bath at 50°C under nitrogen.

Saponification

2ml of 20% potassium hydroxide in ethylene glycol was added to the residue, which was dissolved by progressive heating with mixing and hydrolysis was carried out under reflux at 220°C for 20 minutes. (The upper part of the test tube serves as an air condenser). 10ml of 20% aqueous sodium chloride and 1ml of methanol were added to the cooled

sample containing the deconjugated bile acids.

Extraction of Neutral Sterols

The unsaponifiable matter was extracted from the alkaline mixture by vigorous mixing with 4 x 5 ml of petroleum ether (60-30° petroleum spirit). The petroleum extracts were discarded.

Extraction of Bile Acids and Methylation

The lower layer remaining after extraction with petroleum ether was acidified with 6NHCL to pH 1 or 2 (indicator paper) and the bile acids extracted with 3 x 5 ml diethyl ether. The pooled ether extracts were then evaporated under nitrogen with the succesive addition of toluene (3 x 2 ml).

The dry residue was redissolved in a mixture of diethyl ether (2ml) and methanol (5 drops). An excess of etheral diazomethane was added to give a visible yellow colour and after 15 minutes at 4° C the mixture was evaporated to dryness.

Diazomethane was freshly prepared as follows:
11g of N-methyl-N-nitrosotoluene-4-sulphonamide was added to 15 ml of 50% aqueous potassium hydroxide plus 100 ml ethanol, and distillation carried out at 90-100°C. The distillate was collected in 40 ml of ice cold ether. 10ml of 20% aq NaCl was then added to the residue and the bile acid methyl esters extracted with 3 x 5 ml diethyl ether. The ether was evaporated after addition of toluene (2ml, and the residue transferred to a small tube with 1-2 ml acetone prior to GLC analysis.

GLC Analysis

This was carried out at 275°C using a dual column Pye-104 GLC with flame-ionisation detectors and nitrogen carrier gas. 6' x 4 mm glass columns packed with 2% OV-17on 100-120 mesh Gas chrom Q (Phase-Separations, Flintshire) were used.

Bile Acid Standards

Duplicate standards containing 300 µg/ml of each individual bile acid and the internal standard, 23-norde-oxycholic acid, were taken through the method with each batch of faecal samples analysed.

The bile acids included in the standards were lithocholic, deoxycholic, chenodeoxycholic, cholic and hyodeoxycholic acids.

CALCULATION

The amount of each bile acid in the sample aliquot analysed was calculated by normalising the internal standard peak area (measured by triangulation) in each sample with that in the standards and then comparing the area of each individual bile acid peak in the sample and standards.

The amount of an individual bile acid in the sample (mg)

$$= \frac{\text{mg I.S. sample} \times \text{mg BA std}}{\text{mg I.S. std}} \times \frac{\text{PA I.S. std}}{\text{PA I.S. sample}} \times \frac{\text{PA BA samp}}{\text{PA BA std}}$$

where PA = Peak Area
I.S. = Internal Standard
BA = Individual bile acid

The results for each individual bile acid were summed to give total bile acids in the sample.

Daily faecal bile acid excretion was then calculated as follows.

For human samples

$$\text{mg/day} = \text{mg BA found} \times \frac{H}{A} \times \frac{D}{S} \times \frac{1}{d}$$

where H = total weight of homogenate

A = wt of homogenised aliquot

D = wt of dried aliquot

S = wt of dried aliquot analysed

d = no. of days of collection

For rat samples

$$\text{mg/day} = \text{mg BA found} \times \frac{\text{wt of dried faeces}}{\text{wt of faeces analysed}} \times \frac{1}{7}$$

REPRODUCIBILITY OF METHOD

The variability of results for total faecal bile acid estimations was assessed by analysing samples in duplicate in the same batch of assays and in separate batches. Samples were chosen to cover a wide range of values (Table 5.1). The coefficient of variation (CV) was calculated as follows:

$$CV\% = \frac{\sqrt{\frac{S(d)^2}{2N}}}{\text{Mean}} \times 100$$

where S (d) = sum of differences between duplicates.

N = number of paired observations

For between batch variation the CV was 12.8% and within batch it was 11.4%. These figures were considered acceptable for a long complicated assay procedure such as faecal bile acid determination.

COMPOSITION OF FAECAL BILE ACIDS

Four main peaks were found in the analysis of human faecal bile acids corresponding to lithocholic, deoxycholic, chenodeoxycholic and cholic acid standards, but these peaks were not necessarily homogenous. The cholic acid peak, for example, includes the dihydroxy monoketo derivatives. Other minor unidentified peaks were also found in samples from some patients.

A quantitatively important fifth peak was present in all rat samples and was found to correspond to hyodeoxycholic acid when the authentic compound was injected into the GLC. Thin layer chromatographic analysis had initially revealed a spot with the mobility of hyodeoxycholic acid (Hofmann, 1964) which was confirmed when the compound was obtained (Steraloids Ltd. Croydon).

Although hyodeoxycholic acid had been reported to occur in the rat (Nakita and Wells, 1963; Einarsson, 1966; Demarne et al, 1974), one of the first detailed reports of its quantitative significance in rat faeces was that of Madsen et al in 1976.

Bile acids of the muricholate series could not be obtained commercially but eventually a small sample of β-muricholate was obtained as a gift from Dr. H. Eyssen of the Rega Institute, Louvain, Belgium. This bile acid was found to elute with the cholic acid peak in the GLC system used. A detailed analysis of faecal bile acid composition was considered beyond the scope of these studies and has not been attempted, since mass spectrometry facilities and the necessary authentic bile acid standards were not available.

DISCUSSION

Chromatographing bile acids as the underivatised methyl esters presents problems since cholate is easily absorbed on the column, as indicated by tailing of the peak. With careful selection of batches of OV-17 packing and monitoring of the columns, it was found that a reasonably linear response could be obtained over a limited range. The reproducibility of the method as judged by the between-and within-batch coefficients of variation was considered adequate for the study of gross changes induced by elemental diet feeding.

The original method of Evrard and Janssen which used keto derivatives didn't allow separation of the secondary bile acid deoxycholic from the primary chenodeoxycholic acid, which is an important requirement for human studies. Acetate derivatives were investigated but cholate and hyodeoxycholate could not be separated on OV-17. An alternative liquid phase, PPE-20, was tried and good separation of cholic and hydodeoxycholic acetates was achieved but lithocholic didn't separate from the internal standard.

The advantage of using 23-nordeoxycholic acid is that it is a true internal standard throughout the procedure and does not occur naturally in man or the rat. Other standards that have been used for GLC quantitation may occur naturally and include 7-ketolithocholate (Jones et al, 1976). The Grundy method utilizes both a radioactively labelled recovery marker and 5α cholestane as GLC standard, rather than one internal standard throughout.

For routine clinical samples we are now chromatographing the bile acid methyl ester acetates on OV-17 columns, although a problem was encountered initially with an unidentified extra peak in some patients' samples. This has the mobility of ursodeoxycholic acid and occurs mainly in samples from patients with Crohn's disease. It elutes with chenodeoxycholate as the underivatised methyl ester but separates as the acetate. Significant amounts of ursodeoxycholate have been reported in bile from patients with Crohn's disease (Vantrappen et al, 1977).

TOTAL FAECAL BILE ACIDS, mg/24h			
Within batch variation		Between batches	
A	B	A	B
149	202	276	225
773	743	253	258
331	370	144	168
84	87	57	45
294	272	11	12
376	227	272	308
23	17	772	745
132	193	276	279
40	39	800	758
80	67	1456	1400
310	280	4474	5199
1170	1180	5260	5548
59	51	4432	5154
33	28		
146	105		
170	98		
55	51		
146	147		
1026	947		
232	216		
Coefficient of Variation = 11.4%		Coefficient of Variation = 12.8%	

TABLE 5.1

REPRODUCIBILITY OF FAECAL BILE ACID ESTIMATION

Samples were analysed in duplicate (A and B) in the same batch or in two separate batches. Total faecal bile acids expressed as mg/24h are presented here.

CHAPTER 6

ELEMENTAL DIET FEEDING IN RATS AND TOTAL FAECAL BILE ACIDS

INTRODUCTION

It has been known for many years that dietary manipulation, especially of the fibre content, influences bile acid excretion. Antonis and Bersohn in 1962 showed that the type of fat and also the fibre content of the diet altered the faecal bile acid excretion of South African white and Bantu prisoners. Volunteers living on a low-fat solid diet were found to have a reduced faecal concentration of both bile acids and neutral steroids (Hill, 1971).

The effect of the elemental diet Vivonex on faecal steroid concentration was studied in three normal volunteer subjects by Crowther et al (1973) and faecal bile acid excretion was found to be reduced to an even greater extent than by the use of low-fat solid diets.

Early experiments in rats indicated reduced biliary cholate secretion during semi-synthetic diet feeding in biliary fistula rats (Portman et al, 1955). Later studies using ^{14}C labelled cholic acid showed a reduction in the faecal excretion of cholic acid, calculated indirectly from half-life measurements, when sucrose and starch-based semi-synthetic diets were fed (Portman and Murphy, 1958). Using similar techniques, Gustafsson and Norman also showed that a semi-synthetic starch-based diet resulted in a reduced calculated faecal bile acid excretion in rats (Gustafsson and Norman, 1969a).

The composition of the "semi-synthetic" diets used in these studies was variable and they were not available for

use in humans. The introduction of commercial chemically-defined diets, such as Vivonex and Flexical, for treatment of patients led to the investigation of their direct effects on faecal bile acid excretion. Animal studies were undertaken initially to characterise their effects over a prolonged period and as a result of the difficulty encountered in persuading normal volunteers to consume the rather unpalatable diets.

AIM OF STUDY

To investigate the effects of the elemental diets Vivonex and Flexical on rat growth, faecal weight and total faecal bile acid excretion determined by direct measurement.

METHODS

A preliminary group of rats (Group 1) was fed Vivonex made up into a jelly with 1% agar (as suggested by Dr. A. Ferguson) and showed a marked reduction in faecal weight and total faecal bile acids compared with control rats fed powdered rat chow (Oxoid 41B). When faecal bile acid excretion was expressed in terms of dry weight of faeces, the reduction in the Vivonex fed group was statistically significant for weeks 7 and 9 but not for weeks 3 and 5.

For the second experiment it was discovered, after three weeks, that feeding Vivonex as a liquid, without agar, was acceptable to the rats and no more agar was used. This seemed to have little effect on the bile acid excretion but the further reduction in faecal weight meant that faecal bile acid/g dry wt of faeces was increased in the Vivonex fed rats.

The original period of feeding Vivonex was retained, however, to allow the rats to adjust to the diet and for bile acid metabolism to stabilise.

Collection of Faeces

Rats were housed individually in cages designed to limit coprophagy and allow collection of faeces separately from urine (Figure 6.1). The base of the metal cage was of wide mesh (10 mm spacing) with a lower (3 mm) fine mesh to trap the faecal pellets. Water was supplied from a water bottle and food from a dish in the tunnel at the rear of the cage. The sides of the tunnel were adjusted such that the rats could obtain food without being able to actually enter the tunnel. Thus no faecal matter was lost into the feeding dish. Only one rack containing twelve such cages was available and so the experimental groups were limited in size.

Feeding the Elemental Diets

Control rats were fed powdered chow (Oxoid diet 41B) and Vivonex was fed made up in water (one 30g packet to 125 ml of water). In the later experiments Flexical was diluted similarly (76g to 125 ml water). The composition of the diets is shown in Table 6.1.

Since the availablility of nutrients in the control and elemental diets may be very different, calculation of equivalence as a basis for pair-feeding could not be derived and the rats were fed ad-libitum. Body weight gain was monitored as an indication of comparable growth for the different groups.

Groups Studied

Three groups (Groups 2,3 and 4), each containing six control and six Vivonex fed rats, were studied at different times. Body weight was monitored on a weekly basis and faecal collections for bile acid analysis were made during weeks 3,5,7 and 9. Bile acid half-life and jejunal histology were also examined on these rats as reported in later chapters. Faecal bile acid analysis was performed as outlined in the previous chapter.

A further group of rats (Group 5) was studied as above except that the elemental diet Flexical was used. Unlike Vivonex this diet contains a significant amount of fat.

Statistics

All the analyses were done using a non-parametric ranking test (Wilcoxon's Sum of Ranks Test, as described by Langley, 1963). For convenience however, mean values with standard errors are used in some of the tables for shorter description of the results.

RESULTS

The effect of Vivonex feeding on rat body weight is shown in Table 6.2. After an initial loss of weight, or reduction in weight gain compared with controls, the Vivonex fed rats gained weight satisfactorily. In Group 3 the Vivonex-fed rats were significantly heavier than the controls at weeks 9 and 10 and in Group 4 from week 5 onwards. No initial lag was noted for the rats fed Flexical (Table 6.3).

A dramatic reduction in faecal dry weight was found

during feeding with both elemental diets (Table 6.4). The reduction was similar in all three Vivonex groups, and thus the mean for the three groups together is presented. Both the number and size of faecal pellets was reduced during elemental diet feeding.

The effect of Vivonex feeding on total faecal bile acids for the three groups individually is shown in Table 6.5. There was a significant reduction in total faecal bile acids/100g body weight/week in the Vivonex fed rats compared with the controls in each of the three groups for each week examined. The controls for group 6 were significantly different from those of groups 2 and 4 ($p < 0.01$ in each case) but only group 2 and group 4 Vivonex fed values were significantly different.

When the values for the three groups are combined (Table 6.6) total faecal bile acid excretion for the 13 rats fed Vivonex was significantly less than for the 18 controls for the three weeks studied ($P < 0.002$). (No values are available for group 3, week 5).

Similar results were obtained for the rats fed Flexical (Table 6.7), the reduction in faecal bile acid excretion being significant at weeks 4,5,7 and 9 ($p < 0.01$ in each case). Since the control values for this group are significantly different from the controls in groups 2, 3 and 4 no direct comparison of the effects of Flexical and Vivonex can be made.

DISCUSSION

Normal growth of the rats during feeding with the elemental diets indicated that they were consumed in sufficient quantity to satisfy nutritional requirements and confirms the previous results of the effects of a comparable diet, during preclinical studies, over an eight week period in mature rats (Campbell et al, 1973).

The dramatic reduction in faecal weight during elemental diet feeding which was found is probably due to the absence of fibre in the diets, since feeding Vivonex premixed with methyl cellulose was reported to restore faecal weight to control values (Devereux and Baker, 1977).

The marked reduction in total faecal bile acids during Vivonex feeding in rats is in agreement with the reported studies in three human volunteers (Crowther et al, 1973) and the results obtained during Flexical feeding demonstrates that the effect of fibre is more important in rats than the amount of dietary fat. Flexical, which supplies 30 % of calories as fat, produced a comparable reduction in faecal bile acids to the virtually fat free diet, Vivonex, indicating that the low-fibre content of the elemental diets is very important in relation to bile acid excretion.

This is in agreement with experiments with semi-synthetic diets in rats where bile salt turnover and calculated faecal bile acid excretion was reduced (Portman et al, 1955) and adding 20 % cellulose to the diet largely overcame this effect (Portman and Murphy, 1958). Similar effects of adding cellulose to starch-based semi-synthetic diets in

germ-free rats were also found (Gustafsson and Norman, 1969a). Anotonis and Bersohn (1962) demonstrated that adding 15g of fibre to a low-fat diet increased faecal bile acid excretion in man compared with 4g of fibre.

The effect of the type and amount of fat in the diet on faecal bile acids is less clear, and in man the response to different amounts of fat may be influenced by the fibre content of the diet (Antonis and Bersohn, 1962).

Low-fat solid diets have been shown to reduce faecal bile acid excretion in man (Hill, 1971) but in other studies diets containing 3g and 75 or 100g of saturated fat produced virtually identical faecal bile acid excretion (Gordon et al, 1957).

The results of this study demonstrate that the low-fibre nature of Vivonex may be more important than its low fat content in reducing faecal bile acid excretion in rats since a comparable reduction was found with Flexical, which contains 15% fat by weight.

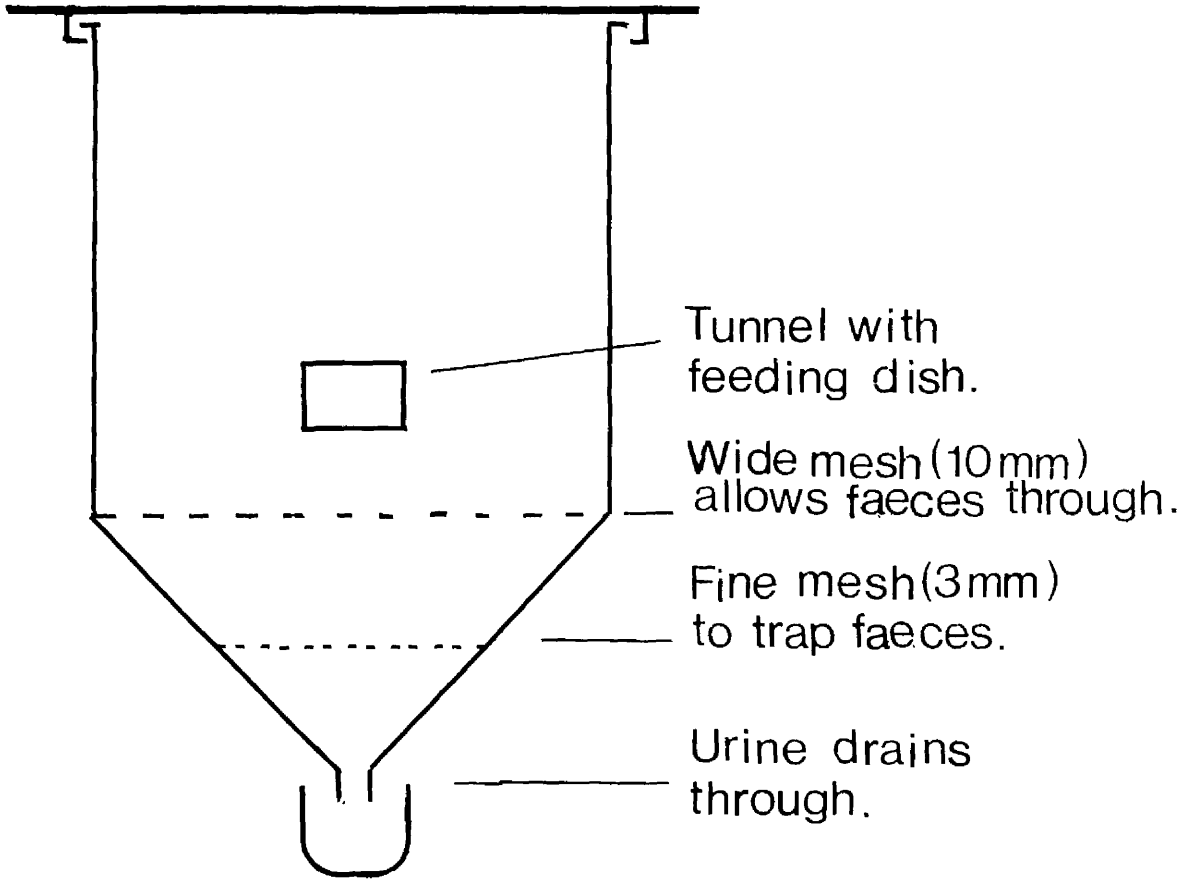


FIGURE 6.1. DIAGRAM OF CAGE USED FOR COLLECTION OF RAT FAECES

Control Diet (Oxoid 41B)

Prepared from:	wholemeal flour	46% w/w
	Sussex ground oats	40%
	white fish meal	8%
	Plus vitamin and mineral supplements	
Supplies:	Crude protein	16.3%
	Crude fibre	5.1%
	Ether extractable lipid	3.0%

Vivonex	Nitrogen as pure amino acids	1.24% w/w
	Fat (purified Safflower oil)	0.54%
	Carbohydrate as glucose solids (73% as pentasaccharides)	84.98%

(Recommended daily intake = 6 x 80g packets)

Flexical	Protein equivalent (70% free aa, 30% small peptides)	9.9% w/w
	Fat (20% MCT, 80% soy-oil)	15.0%
	Carbohydrate (65.5% sucrose 31.4% glucose oligosaccharides 3% citrate).	67.8%

(Recommended daily intake = 454g)

TABLE 6.1

COMPOSITION OF CONTROL RAT DIET, VIVONEX AND FLEXICAL

Start of Week No.		1	2	3	4	5	6	7	8	9	10
Group 2	C	203 ± 10	207 ± 12	253 ± 9	267 ± 8	286 ± 4	304 ± 4	325 ± 4	343 ± 6	346 ± 5	376 ± 7
	V	206 ± 11	199 ± 10	224 ± 10	237 ± 12	246 ± 10**	292 ± 8	332 ± 8	355 ± 11	362 ± 10	392 ± 12
Group 3	C	160 ± 3		210 ± 3	243 ± 5	263 ± 5		275 ± 6		319 ± 2	342 ± 5
	V	160 ± 3		198 ± 3	243 ± 3	271 ± 5		297 ± 6		348** ± 6	365* ± 6
Group 4	C	203 ± 4	251 ± 11	276 ± 4	305 ± 11	317 ± 4	325 ± 4	328 ± 5	355 ± 6	363 ± 6	376 ± 6
	V	203 ± 5	241 ± 6	264 ± 2	314 ± 2	336* ± 4	338* ± 5	356* ± 6	392** ± 6	412** ± 8	428** ± 8

* $p < 0.05$ Si nificance of difference between
** $p < 0.01$ control and Vivonex fed rats.

TABLE 6.2 EFFECT OF VIVONEX FEEDING ON RAT BODY WEIGHT (g)

MEAN ± SEM C = CONTROL (n=6) V = VIVONEX (n=6)

Start of Week No.	1	2	3	4	5	6	7	8	9	10
Control (n=6)	192 ± 5	222 ± 5	258 ± 6	297 ± 6	316 ± 13	349 ± 6	373 ± 8	394 ± 8	409 ± 8	419 ± 8
Flexical (n=6)	193 ± 3	232 ± 4	281* ± 6	300 ± 7	323 ± 8	366 ± 8	399 ± 9	427* ± 7	434 ± 7	423 ± 6

* $p < 0.05$ Control and Flexical significantly different

TABLE 6.3 EFFECT OF FLEXICAL FEEDING ON RAT BODY WEIGHT (g)

Group 5 Rats. Mean ± SEM

1. Vivonex. Combined Results for Groups 2,3 and 4

	Dry weight of faeces (g/week)	
	Control (n=18)	Vivonex (n=18)
Week 3	56.9 ± 5.4	2.72 ± 0.38
Week 5	44.3 ± 1.2	1.40 ± 0.06
Week 7	57.1 ± 3.4	1.95 ± 0.06
Week 9	57.1 ± 3.0	2.05 ± 0.11

Control v Vivonex $p < 0.002$ throughout

2. Flexical. Group 5

	Dry weight of faeces (g/week)	
	Control (n=6)	Flexical (n=6)
Week 3	47.3 ± 1.8	2.73 ± 0.07
Week 5	58.9 ± 1.9	3.51 ± 0.10
Week 7	50.9 ± 1.3	3.59 ± 0.10
Week 9	46.7 ± 0.9	2.15 ± 0.04

Control v Flexical, $p < 0.01$ throughout

TABLE 6.4

EFFECT OF ELEMENTAL DIET FEEDING ON RAT FAECAL WEIGHT

(Mean ± SEM)

	Total faecal bile acids (mg/100g body weight/week)			
	Week 3	Week 5	Week 7	Week 9
Group 2				
Controls (n=6)	± 31.20 ± 3.74	± 38.36 ± 2.87	± 35.38 ± 1.27	± 35.42 ± 2.47
Vivonex (n=6)	± 15.57* ± 3.35	± 10.29 ± 1.38	± 7.24 ± 1.30	± 4.39 ± 1.04
Group 3				
Controls (n=6)	± 45.99 ± 3.99		± 44.73 ± 3.07	± 41.37 ± 2.99
Vivonex (n=6)	± 13.94 ± 2.33		± 12.68 ± 1.84	± 10.23 ± 2.01
Group 4				
Controls (n=6)	± 28.13 ± 3.28	± 22.39 ± 1.06	± 29.72 ± 2.87	± 24.24 ± 1.54
Vivonex (n=6)	± 4.15 ± 0.58	± 3.31 ± 0.49	± 3.34 ± 0.30	± 5.04 ± 0.07

* $p < 0.05$. All other Control v Vivonex comparisons, $p < 0.01$

TABLE 6.5

EFFECT OF VIVONEX ON RAT FAECAL BILE ACID EXCRETION

(Mean ± SEM)

	Total faecal bile acids (mg/100g body weight/week)		
	Week 3	Week 7	Week 9
Control (n=13)	35.11 ± 2.75	36.61 ± 2.04	33.67 ± 2.16
Vivonex (n=13)	11.22 ± 1.94	7.75 ±1.17	6.55 ± 0.93

Control v Vivonex, $p < 0.002$ in each case

TABLE 6.6

EFFECT OF VIVONEX ON RAT FAECAL BILE ACID EXCRETION:

COMBINED RESULTS FOR THE GROUPS 2, 3 AND 4. (Mean ± SEM)

	Total faecal bile acids (mg/100g body weight/week)			
	Week 4	Week 5	Week 7	Week 9
Control (n=6)	21.38 ± 0.51	32.90 ± 5.13	23.62 ± 1.55	17.44 ± 1.25
Flexical (n=6)	4.37 ± 0.33	5.43 ± 0.38	7.58 ± 1.17	5.64 ± 0.44

Control v Flexical, $p < 0.01$ in each case.

TABLE 6.7

EFFECT OF FLEXICAL ON RAT FAECAL BILE ACID EXCRETION

(Mean ± SEM)

CHAPTER 7

CHANGES IN THE COMPOSITION OF RAT FAECAL BILE ACIDS INDUCED BY ELEMENTAL DIET FEEDING

INTRODUCTION

Transformation of bile acids by micro-organisms occurs only to a minor extent in the rat small intestine, extensive transformation by microbial enzymes starting in the caecum and continuing in the colon (Norman and Sjövall, 1953). Various quantities of the metabolites formed are absorbed in the caecum and colon and transported to the liver where they may be reconjugated and rehydroxylated (Bergström et al, 1960; Thomas et al, 1964).

The composition of faecal bile acids depends upon the nature of the microbial population of the gut; the amount of bile acid absorption in the small intestine, caecum and colon; the transit or contact time and the number of enterohepatic circulations before final elimination in the faeces.

Prolonged retention of carmine marker was demonstrated in rats fed semi-synthetic diets (Gustafsson and Norman, 1969a) and diet has been shown to alter the composition of gut microflora (Gall et al, 1948).

Elemental diet feeding in humans was reported to drastically reduce the microbial population of faeces (Winitz et al, 1966; Winitz et al, 1970) but these reports were not confirmed in later studies and the techniques used were questioned. Changes in the composition of the faecal flora were found, however, with a reduction in enterococci and increase in enterobacteria (Attebury et al, 1972; Drowther et al, 1973; Bounous and Devroede, 1974).

Crowther and co-workers also reported a reduction in the degradation of neutral sterols during Vivonex feeding but did not examine the composition of the acid sterol fraction. However, Gustafsson and Norman (1969a), found a reduction in the percentage of monohydroxy-monoketo-cholanoic acids in rats fed a semi-synthetic starch-based diet.

AIM OF STUDY

To investigate the influence of elemental diet feeding, particularly Vivonex, on the composition of faecal bile acids in the rat.

METHODS

Faecal bile acids were determined as outlined in Chapter 5. A total of 13 control and 13 rats fed Vivonex were studied in groups 2, 3 and 4, as described in the previous chapter, together with 6 control and 6 Flexical fed rats.

The absolute amount of individual bile acids was expressed as mg/100g body weight/week and the percentage composition of the faecal bile acids was calculated, for each rat, by dividing the amount of a given bile acid excreted by the total amount of bile acid excreted and expressing as a percentage.

Statistical Analysis

Differences between control and treated groups were analysed by non-parametric ranking tests (Langley, 1963) but for ease of presentation of data mean values are included in the tables.

Abbreviations Used

LC - Lithocholic acid and any other bile acids eluting in the same peak under the conditions employed for gas-liquid chromatographic analysis.

Similarly:

DC - Deoxycholic acid and other bile acids included in the peak.

CDC - Chenodeoxycholic acid peak (includes ursodeoxycholic acid if present).

HDC - Hyodeoxycholic acid peak.

C - Cholic acid peak which also includes B-muricholate and the dihydroxymonoketo derivatives (7-keto and 12-keto) and probably other trisubstituted 5β-cholanic acids.

RESULTS

The absolute amounts of the five bile acids (or five groups of bile acids) excreted by the control (n=13) and Vivonex fed (n=13) rats are shown in Table 7.1. The small amounts of chenodeoxycholic acid excreted meant that it was detected in some rats and not others. Excretion of LC, DC and HDC was significantly reduced in the Vivonex fed rats for the three weeks studied ($p < 0.002$ in each case), but the amount of cholic acid was not significantly reduced.

This is reflected in the relative proportions of individual bile acids excreted (Table 7.2) and the % contribution of the C peak was in fact increased in the Vivonex fed rats. The increase is significant for weeks 3 and 9 ($p < 0.01$). The proportion of LC and DC was markedly reduced in the rats fed Vivonex for all three weeks ($p < 0.002$ in all cases) and the proportion of HDC unchanged.

Within groups, there was wide variation between rats (both control and Vivonex fed) in the relative amounts of HDC and C. Four control rats in group 4 had 48-56% HDC and one control rat in group 3 excreted 59% HDC. The controls for the Flexical group were all found to excrete a high % of HDC, which together with the small number of Flexical fed rats studied, makes comparison of the effects of Vivonex and Flexical in relation to the excretion of HDC and C meaningless.

The results for Flexical feeding are shown in Tables 7.3 and 7.4. There was a significant reduction in the amount of LC, DC and HDC excreted in the Flexical fed rats and, in contrast to the Vivonex fed group, the amount of C was also reduced ($p < 0.01$ throughout). The small numbers and variability of results make interpretation of the % composition results difficult, except that the % of LC was probably reduced ($p < 0.01$ for weeks 7 and 9).

DISCUSSION

The heterogeneity of the GLC peaks, especially of the 'cholic' peak, and the difficulty in obtaining pure compounds of the muricholate series has limited the scope of this investigation of the effect of elemental diet feeding on the composition of faecal bile acids in the rat.

A recent report (Madsen et al, 1976 suggests that hyodeoxycholic and w-muricholic acid (Madsen et al, 1975) comprise 50% of the faecal bile acids excreted by the conventional rat. No w-muricholate was available and so its behaviour in the GLC system used is not known, but it

is probably included in the cholic peak along with β-muricholate. C and HDC did account for 50% of total faecal bile acids for the controls in this study.

In the rat hyodeoxycholate and the muricholate series arise by a combination of bacterial action in the gut and 6 β hydroxylation in the liver (Einarsson, 1966; Mitropoulous and Myant, 1967; Madsen et al, 1975). Deoxycholic and lithocholic acid can also be rehydroxylated at the 7α position in the rat liver (Bergström et al, 1960; Thomas et al, 1964) so there is no simple relationship of primary (synthesised in liver) to secondary (products of bacterial action on primary) bile acids in the rat.

The LC and DC peaks, including mono and di-substituted bile acids, do however represent bacterially degraded bile acids which indicates that Vivonex feeding reduces not only the amount but also the proportion of bacterially degraded bile acids in rat faeces. Although the relative contributions of cholic, β-muricholate and other bile acids to the C peak is not known, and the proportions may change during elemental diet feeding, these all represent less degraded products of cholic acid, the main primary bile acid in the rat (Danielson, 1963). It therefore appears that bacterial modification of faecal bile acids is reduced during Vivonex feeding since both the proportion of trisubstituted bile acids is increased and the proportions of LC and DC are reduced.

Overall transit time of labelled sodium dichromate marker is increased in rats fed Vivonex, (Devereux and Baker, 1977), but whether this results from prolonged transit

time throughout the GI tract or selectively small or large bowel is unknown. It is possible that less bile acid reaches the caecum and colon for bacterial modification as a result of more efficient absorption in the ileum, but the reduced proportion of LC and DC suggests that either the overall number of micro-organisms is reduced or their composition is altered during Vivonex feeding.

Faecal bile acid excretion during Vivonex feeding is analogous to that of germ free rats (Kellogg and Wostmann, 1969; Madsen et al, 1976) in that total excretion is reduced and the proportion of lithocholate and deoxycholate decreased. Cholate and β-muricholate were reported to comprise 96% of the faecal bile acids in germ-free rats (Madsen et al, 1976) which is consistent with the increased proportion of the C peak in the rats fed Vivonex.

The results for Flexical feeding cannot be compared with those for Vivonex since the controls for the two groups are different and the numbers in the Flexical group are small. However, the proportion of lithocholate was apparently reduced during Flexical feeding.

	Bile acid excreted, mg/100g body weight/week (Mean ± SEM)				
	LC	DC	(CDC)	HDC	C
Week 3 C	4.67 ±0.69	10.57 ± 0.96	1.26	9.80 ± 1.00	8.32 ±1.47
V	0.52 * ±0.33	1.50 * ±0.59	0.04	2.17 * ±0.69	6.98 NS ±1.62
Week 7 C	4.82 ±0.31	10.57 ± 0.96	0.52	12.94 ± 1.00	7.43 ±1.36
V	0.06 * ±0.02	0.47 * ±0.07	0.07	1.97 * ± 0.59	5.19 NS ±1.23
Week 9 C	4.06 ±0.44	10.20 ± 0.55	0.25	12.40 ± 1.43	6.22 ±0.92
V	0.07 * ±0.02	0.50 * ±0.09	0.12	1.85 * ±0.45	4.02 NS ±1.03

C = control (n=13) V = vivonex-fed (n=13)

(CDC) - CDC detected in a few rats only. Present in small amounts at limit of detection.

* Control v Vivonex significantly different, $p < 0.002$

NS = not significant

TABLE 7.1

EFFECT OF VIVONEX FEEDING ON EXCRETION OF INDIVIDUAL BILE ACIDS IN THE RAT. COMBINED RESULTS FOR GROUPS 2, 3 AND 4

		% of total faecal bile acids. (Mean ± SEM)				
		LC	DC	CDC	HDC	C
Week 3	C	12.71 ± 1.29	30.07 ± 1.62	4.12 ±1.27	28.95 ± 2.45	23.78 ± 3.02
	V	3.40** ±1.10	13.05** ± 1.83	0.65 ±0.38	26.35NS ± 6.58	56.77* ± 7.34
Week 7	C	13.33 ± 0.71	30.74 ± 1.62	1.57 ±0.87	35.52 ± 2.03	18.84 ± 2.59
	V	1.50** ±0.61	7.81** ±2.20	0.95 ±0.54	35.16NS ± 8.31	54.59NS ± 9.19
Week 9	C	12.35 ± 1.18	32.09 ± 1.83	0	36.20 ± 2.74	18.16 ± 1.97
	V	1.09** ±0.30	7.74** ±1.32	3.91 ±1.83	32.03NS ± 7.18	55.27* ± 7.60

C = control (n=18) V = Vivonex fed (n=18)

Control v Vivonex significantly different * $p < 0.01$
** $p < 0.002$

NS = not significant

TABLE 7.2

EFFECT OF VIVONEX FEEDING ON COMPOSITION OF RAT FAECAL BILE ACIDS. COMBINED RESULTS FOR GROUPS 2, 3 AND 4

		Bile acid excreted.mg/100g body weight/week(Mean±SEM)				
		LD	DD	CDD	HDC	C
Week 4	C	1.33 ±0.17	3.92 ±0.26	2.33 ±0.50	9.65 ±0.51	3.70 ±0.86
	F	0.30* ±0.05	1.15* ±0.11	0.35 ±0.10	1.29* ±0.30	1.27* ±0.32
Week 7	C	1.33 ±0.21	4.52 ±0.27		12.10 ± 1.44	5.66 ±1.46
	F	0.13* ±0.04	1.29* ±0.13		4.71* ±1.24	1.33* ±0.21
Week 9	C	1.23 ±0.13	5.24 ±0.22		8.84 ±0.94	2.08 ±0.36
	F	0.15* ±0.03	1.17* ±0.09		3.89* ±0.40	0.43* ±0.09

C = control (n=6) F = Flexical fed (n=6)
* $p < 0.01$ for control v Flexical

TABLE 7.3

EFFECT OF FLEXICAL FEEDING ON THE EXCRETION OF INDIVIDUAL BILE ACIDS IN THE RAT.

		% of total faecal bile acids (Mean ± SEM)				
		LC	DC	CDC	HDC	C
Week 4	C	3.76 ±0.69	13.37 ± 1.15	10.49 ± 2.29	45.21 ± 2.35	7.13 ±3.76
	F	6.86 ±0.91	26.79* ± 2.58	7.37 ±2.02	29.79 ± 6.19	23.58 ± 5.65
Week 7	C	5.92 ±1.34	19.31 ± 0.83		51.36 ± 5.22	23.41 ± 5.30
	F	1.42* ±0.45	13.04 ± 2.16	3.03 ±3.03	53.94 ± 9.94	21.62 ± 6.61
Week 9	C	7.33 ±0.46	30.59 ± 1.39		50.28 ± 2.47	11.30 ± 1.82
	F	2.58* ±0.24	21.02* ± 1.65		68.69* ± 2.57	7.73 ±1.73

C = control (n=6) F = Flexical fed (n=6)

* $p < 0.01$ for control v Flexical.

TABLE 7.4

EFFECT OF FLEXICAL FEEDING ON THE % COMPOSITION OF RAT FAECAL BILE ACIDS.

CHAPTER 3

THE EFFECT OF VIVONEX AND FLEXICAL ON BILE ACID KINETICS IN THE RAT

INTRODUCTION

The reduced faecal bile acid excretion observed in the rats fed elemental diets could result from either a reduced body pool of bile acids or reduced turnover of a normal or even increased pool. Cholate half-life was reported to be increased in rats fed starch and sugar based semi-synthetic diets (Portman and Murphy 1953; Portman 1960; Gustaffson and Norman, 1969 a) but the reported effects on cholate pool size are conflicting. Other studies in biliary fistula rats suggested that sugar based diets inhibit the synthesis of bile acids from cholesterol, thus reducing the cholate pool (Portman et al, 1955; Lee and Herrmann, 1963).

AIM OF STUDY

To investigate the effects of Vivonex and Flexical on the biological half life of ^{14}C labelled cholic acid and on total liver cholesterol levels in rats, and also on the estimated bile acid pool size.

METHODS

Groups Studied

Cholic acid half life was measured in the rats from Groups 3 and 4 (Vivonex studies) and Group 5 (Flexical) after two months. The rats were then sacrificed and liver cholesterol measured.

Measurement of Cholic Acid Half Life

This was performed according to the method of Lindstedt and Norman (1956) as described by Strand (1963) and Gustafsson and Norman (1969a).

On day 1 of the experiment, 4.5μCi of ^{14}C cholic acid (Radiochemical Centre, Amersham) was injected intraperitoneally into each rat and daily faecal collections were then made

for nine days. Urine collections were also checked for radioactivity and found to contain negligible amounts as expected (Gustafsson and Norman, 1969a; Behar et al, 1969). The daily excretion of isotope was determined using a sample oxidiser as outlined below and the half life of cholic acid was calculated from the plot of -loge (1-Ut/Umax) against time.

Where U_t = cumulative amount of isotope excreted to time t

and U_{max} = administered amount of isotope.

The best-fit straight line was determined by the method of least squares and the intercept, slope and correlation coefficient determined using a programmable calculator and linear regression programme (Wang 600 series General Program Library, Wang Laboratories Inc.).

The biological half life of cholic acid, which is the time taken for half of the administered dose to be excreted, was calculated from the slope of the semi-logarithmic plot.

$$t\tfrac{1}{2} = \frac{0.693}{k}$$ where $0.693 = \log_e 2$

and k = slope of line (decay constant)

Estimation of Bile Acid Pool Size

An estimate of bile acid pool size (mg/100g body weight) was obtained from the total faecal bile acid excretion during week 9 of the study (see Chapter 6), expressed as mg/100g body wt/day, and the cholic acid half life (days) using the relationship:

$$\text{Bile acid excretion} = \frac{0.693}{t\tfrac{1}{2}} \times \text{pool size}$$

Daily faecal weight for the rats fed the elemental

was too small to allow determination of mass and radioactivity on the one collection and so the week 9 values for faecal bile acid excretion were used. This pool size estimate makes the assumption that cholic acid turnover is of the same order of magnitude as that of chenodeoxycholate, as has been demonstrated for conventional rats fed commercial rat diets (Lindstedt and Norman, 1956), but may not be true during elemental diet feeding.

Determination of Isotope (^{14}C) in Rat Faeces Using a Sample Oxidiser

(a) Principle of operation.

An IN 4101 automatic sample oxidation unit for liquid scintillation counting (Intertechnique Ltd) was used. ^{14}C labelled samples are prepared for liquid scintillation counting by catalysed combustion to $^{14}CO_2$ in oxygen at 700°C. The $^{14}CO_2$ is absorbed and eluted in scintillant ready for scintillation counting.

Scintillant composition:		
	Phenylethylamine	330 ml
	Methanol	220 ml
	Toluene	400 ml
	Distilled H_2O	50 ml
	Butyl-PBD	7 g

(b) Sample Preparation.

Daily faecal samples from Vivonex or Flexical fed rats were transferred to combustion capsules for the IN 4101 after drying overnight. In most cases the dried faecal weight was less than 0.5g and the entire sample could be combusted in one capsule. Larger samples were transferred to two capsules.

Daily faecal samples from the control rats were dried, weighed and powdered in pestle and mortar. Approximately 0.3 g was then accurately weighed into an IN 4101 capsule.

(c) Liquid Scintillation Counting.

This was performed using a Packard Tri-carb liquid scintillation counter equipped with automatic external standardisation. Quench curves were prepared using internal standards in a number of oxidiser samples from each day's batch and correlating with the external standard ratio. Counting efficiency was 40-60%.

(d) Oxidiser Performance.

The recovery of ^{14}C from labelled cholic acid was checked by comparing oxidised standards with standards transferred directly to scintillation vials, which were then used for a blank run of the oxidiser. Recovery was always greater than 95%.

Memory effects were checked by burning a blank capsule immediately after a radioactive standard. Memory was found to be less than 0.5%. To avoid contamination of low activity samples (control samples, especially of the later days) from high activity samples (elemental diet samples) a wash cycle was always implemented between the different types of samples.

Measurement of Total Liver Cholesterol

Total liver cholesterol was measured using a modified Liebermann-Burchard colour reagent (Abell et al, 1952), and based on the measurement of liver cholesterol described by Herrmann (1957).

Duplicate aliquots (0.25g) of frozen liver were weighed into tubes and homogenised in water (0.5 ml) with a hand operated teflon pestle. The homogenate was transferred to a stoppered 20ml tube with 5 ml freshly prepared alcoholic potassium hydroxide (6ml of 33% aqueous KOH + 94ml absolute ethanol). The tubes were mixed well and incubated in a water bath at 37-40°C for 55 minutes. After cooling to room temperature, 20ml of n-hexane was added and mixed well. 5ml of water were then added and the tubes shaken vigorously for one minute. When the phases had separated two aliquots of the upper organic phase, usually 3ml and 5ml, were transferred to 10ml stoppered tubes and the solvent evaporated under nitrogen.

Duplicate 5ml samples of cholesterol standard solution (0.58 mg/ml in absolute ethanol; prepared by diluting stock 5.8 mg/ml cholesterol standard from B.D.H. Ltd.) were mixed with 0.3ml of 33% KOH, incubated and extracted with the samples, and aliquots of the hexane layer (1, 2 and 3 ml) evaporated as for the samples. The modified Lieberman-Burchard colour reagent was prepared as follows:

> 20vol of acetic anhydride were chilled to less than 10°C and 1vol of conc H_2SO_4 added. The well-shaken mixture was kept cold for 3 min, then 10vol of glacial acetic acid were added and the mixture warmed to room temperature. The reagent was used within one hour of preparation.

All sample and standard tubes plus blank tubes were placed in a 25°C water bath and 6ml of the colour reagent added to each at timed intervals (30 secs) with vigorous shaking.

After exactly 30 min. the colour development was read at 620nm (Gilford 300N spectrophotometer). Care was taken to protect the samples from intense light during colour development.

Total liver cholesterol was expressed as mg/100g wet weight of liver.

Statistics

The significance of differences between groups of control and elemental diet fed rats was analysed using Wilcoxon's sum of ranks test (Langley, 1968).

RESULTS

A linear relationship between $\log_e$ (1-Ut/U max) and time was found for the excretion of ^{14}C labelled cholic acid in all the rats studied. The correlation coefficients for the regression analysis are shown in Table 3.1, and were all highly statistically significant ($p < 0.002$ in all cases) (Colton, 1974).

The half-life of cholic acid determined from the slope of the semi-logarithmic plots (Table 3.2) was found to be significantly increased from 2.313 $\pm$ 0.227 days in the controls (n=12) to 9.913 $\pm$ 0.855 in the rats fed Vivonex (n=12); $p < 0.002$. For the rats fed Flexical (n=6) cholic acid half-life was 6.451 $\pm$ 0.551 days which was significantly greater than 1.572 $\pm$ 0.039 days for the controls (n=6), $p < 0.01$.

There was no significant difference in cholic acid half-life between the controls for Groups 3 and 4 or the Vivonex fed rats of Groups 3 and 4. The controls for Group 5, the Flexical group, were not significantly different from the

controls for Group 4 (although significantly different from the combined controls for Groups 3 and 4) so that the results for Vivonex and Flexical feeding can be tentatively compared using these two groups. Cholic acid half-life in the Vivonex fed rats of Group 4 (n=6) was 10.734 $\pm$ 1.159 days compared with 6.431 $\pm$ 0.551 in the Flexical fed rats (n=6). This difference was statistically significant ($p < 0.02$).

Estimated bile acid pool size for the control rats and those fed the elemental diets was not significantly different as shown in Table 3.3. The mean ($\pm$ SEM) bile acid pool size for the controls in the Vivonex study was 16.53 $\pm$ 2.31 mg/100g body weight and for the Vivonex fed rats, 14.53 $\pm$ 2.22. In the Flexical study the controls had a mean pool size of 5.67 $\pm$ 0.57 mg/100g body weight compared with 7.64 $\pm$ 1.05 for the Flexical fed rats.

The effects of elemental diet feeding on total liver cholesterol is shown in Table 3.4. Total liver cholesterol (Mean $\pm$ SEM) was significantly increased from 220.4 $\pm$ 2.2 mg/100g for the controls (n=12) to 381.6 $\pm$ 33.1 in the rats fed Vivonex (n=12), ($p < 0.002$) and from 241.9 $\pm$ 4.1 in the controls (n=6) to 265.6 $\pm$ 8.8 in the Flexical fed rats (n=6), ($p < 0.01$). The difference between controls means that the effects of Vivonex and Flexical on bile acid pool size and cholesterol levels cannot be compared directly.

DISCUSSION

A good linear relationship was obtained for the semi-logarithmic plot of cholic acid excretion against time, as previously described (Strand, 1963; Gustafsson and Norman, 1969 (a); Beher et al, 1969), indicating that the excretion was governed by first-order kinetics.

The values obtained for cholic acid half-life in the control rats are in good agreement with previously reported values using different methods for determining the isotope excreted in the faeces. The values reported include a mean cholate half-life of 2.0 days with a range of 1.55 - 2.51 days using ethanolic extraction of faeces (Portman and Murphy, 1953); a mean half-life of 2-3 days, range 1.7-2.9 days by combustion in a Schöniger flask (Strand, 1963); a half life 2.5-3.5 days by extraction (Gustafsson and Norman, 1969a) and a half life of 3.5 $\pm$ 0.4 days (Mean $\pm$ SEM) by both extraction and combustion methods (Beher et al, 1969).

The increase in half life of cholate produced by elemental diet feeding is similar to that reported for semi-synthetic diets. Cholate half life was increased from 2.0 days to 3.2 days by starch based, and 4.2 days by sucrose based, semi-synthetic diets after four weeks feeding (Portman and Murphy, 1953). Lactose based diets were reported to increase cholate half life to 6.5 days (Portman, 1960) and starch based semi-synthetic diets to increase it to 3-5 days after three weeks (Gustafsson and Norman, 1969a). The latter authors reported a cholate half life for germ-free rats fed the starch based diet of 9-16 days and 4-6 days on pellets. They also demonstrated that prevention of coprophagy had no effect on cholate half life measurements in any of the groups studied. Chemotherapeutic agents prolonged cholate half life on sucrose based diets to 11.4 days (Portman, 1960).

Vivonex feeding for nine weeks resulted in a cholate half life greater than that reported for sucrose diets but comparable to that found in germ-free rats on starch based diets and normal rats fed sucrose diet plus Sulphasuxadine. This long half life may therefore be due to alterations in bacterial flora, to the differences in composition of the diets or the longer feeding period employed in this study. Flexical had a less pronounced effect than Vivonex on cholate half life which may be due to the difference in composition of the diets. The nitrogen source in Vivonex is synthetic amino acids, casein hydrolysate in Flexical and was whole casein in the semi-synthetic diets. Also Flexical contains sucrose but Vivonex doesn't and the fat content is different. The results for faecal bile acid composition in the previous chapter also suggested that Vivonex feeding may produce alterations in bacterial flora.

The reported effects of diet on bile acid or cholic acid pool sizes which are calculated by different methods are conflicting, and many of the methods are questionable. Cholic acid pool size, calculated from the counts injected and the specific activity of cholate in bile 4 hours later (for two rats only for each group), was reported to be reduced by starch and sucrose diets to half the value found in chow fed rats (Portman and Murphy, 1953). A later study, in which cholate pool size was calculated from the specific activity of cholate in the small intestine and the total radioactivity remaining in the rat, showed no significant difference between conventional rats fed pellets and starch based diets, nor between conventional rats fed

pellets and germ-free rats fed the synthetic diet, although the differences in cholic acid half life were marked (Gustafsson and Norman, 1968a). The results of this later more reliable study are in agreement with the results for elemental diet feeding obtained here, in that the bile acid pool size is apparently not significantly altered although great differences in the biological half life of cholic acid were found.

Total liver cholesterol was measured as a preliminary investigation of the effects of elemental diet feeding on the synthesis of bile acids, since early studies with bile fistula rats proposed that bile acid synthesis was inhibited by sugar based diets and resulted in a reduced cholate pool size (Portman et al, 1955; Lee and Herrmann, 1963). The results for liver cholesterol levels in the control rats are in agreement with those reported by Lee and Herrman, and were found to be increased in the rats fed elemental diets. However, since no evidence was obtained that bile acid pool size was reduced there is no need to invoke an inhibition of the conversion of cholesterol to bile acids as the cause of reduced faecal bile acid excretion during elemental diet feeding. In fact, cholesterol accumulation in the liver may simply reflect the fact that bile acid turnover is reduced during elemental diet feeding and so less cholesterol is converted to bile acids to maintain the pool size. Accumulation of rat liver lipid during elemental diet feeding has recently been reported in abstract form (Young et al, 1973). The accumulation was greater during Vivonex feeding than during Flexical feeding as suggested by the results of this study.

Elemental diet feeding in rats increased cholic acid half life and faecal bile acid excretion was reduced. Thus the bile acids excreted represent a smaller proportion of a normal sized bile acid pool rather than reflecting a reduced pool size.

The differences in the amount and composition of faecal bile acids excreted, and also in cholic acid turnover, that exist between the conventional rats fed pellets and elemental diets (especially Vivonex) in this study are similar to the differences observed between conventional and germ-free rats when fed the same diet (Gustafsson et al, 1960; Gustafsson and Norman, 1969 a and b; Kellogg and Wostmann, 1969; Madsen et al, 1976), and so changes in bacterial flora may be implicated. The mechanism of the altered bile acid metabolism in germ-free rats is not fully understood, however, but enhanced absorption of bile acids in the small intestine leading to an increase in the number of entero-hepatic cycles of bile acids has been implicated (Kellogg and Wostmann, 1969).

	Correlation Coefficient	
	Control	Vivonex
Group 3	0.9962	0.9868
	0.9933	0.9647
	0.9988	0.9927
	0.9982	0.9905
	0.9976	0.9860
	0.9887	0.9835
Group 4	0.9943	0.9916
	0.9951	0.9685
	0.9821	0.9922
	0.9652	0.9713
	0.9925	0.9830
	0.9964	0.9935
	Control	Flexical
Group 5	0.9512	0.9817
	0.9515	0.9889
	0.9991	0.9817
	0.9957	0.9930
	0.9715	0.9809
	0.9975	0.9925

TABLE 3.1

CHOLIC ACID HALF-LIFE DETERMINATION: CORRELATION COEFFICIENT FOR PLOT OF $-LCC_e(1-U_t/U_{max})$ VERSUS TIME

/

	Cholic acid half life (days)		
	Control	Vivonex	
Group 3	2.342	8.178	
	4.109	15.254	
	2.666	3.458	
	2.717	7.492	
	2.529	7.154	
	2.223	7.773	
Group 4	2.819	9.246	
	1.731	16.090	
	1.121	7.942	
	1.247	11.211	
	1.953	9.604	
	2.299	10.610	
Mean ±SEM	2.318 ±0.227	9.918 ±0.855	$p < 0.002$
	Control	Flexical	
Group 5	1.620	4.785	
	1.507	6.179	
	1.374	8.542	
	1.329	5.605	
	1.351	6.030	
	1.752	7.447	
Mean ±SEM	1.572 ±0.089	6.431 ±0.551	$p < 0.01$

TABLE 3.2

EFFECT OF ELEMENTAL DIET FEEDING ON CHOLIC ACID HALF-LIFE IN RATS

	Estimated bile acid pool size (mg/100g body weight)		
	Control	Vivonex	
Group 3	24.10	16.52	
	38.63	9.11	
	19.29	31.04	
	26.33	16.68	
	20.33	18.23	
	14.39	12.34	
Group 4	14.59	9.53	
	7.38	26.54	
	7.21	9.38	
	6.14	10.13	
	9.16	7.12	
	10.66	7.44	
Mean ±SEM	16.53 ± 2.81	14.53 ± 2.22	N.S.
	Control	Flexical	
Group 5	5.34	4.34	
	4.69	7.60	
	5.37	9.33	
	4.96	6.01	
	4.79	6.34	
	8.37	11.67	
Mean ±SEM	5.67 ±0.57	7.63 ±1.05	N.S.

TABLE 3.3

EFFECT OF ELEMENTAL DIET FEEDING ON ESTIMATED BILE ACID POOL SIZE IN RATS

N.S. = Not significant

	Total liver cholesterol (mg/100g wet weight)		
	Control	Vivonex	
Group 3	222.6	273.4	
	222.3	490.5	
	212.8	229.3	
	217.4	293.3	
	223.4	347.1	
	236.6	347.8	
Group 4	223.7	470.6	
	220.0	299.6	
	213.4	593.1	
	226.5	413.3	
	208.0	297.3	
	212.3	523.5	
Mean ±SEM	220.4 ± 2.2	391.6 ±33.1	$p < 0.002$
	Control	Flexical	
Group 5	247.0	265.7	
	252.9	264.9	
	250.9	235.5	
	235.1	260.7	
	227.5	302.9	
	237.9	264.1	
Mean ±SEM	241.9 ± 4.1	265.6 ± 8.3	$p < 0.05$

TABLE 3.4

EFFECT OF ELEMENTAL DIET FEEDING ON

RAT LIVER TOTAL CHOLESTEROL

CHAPTER 9

VIVOREX AND HUMAN FAECAL BILE ACIDS:

MANAGEMENT OF PATIENTS WITH CHOLERIEIC DIARRHOEA

INTRODUCTION

The importance of the ileum for the absorption of bile acids and maintenance of their enterohepatic circulation has been demonstrated in dogs (Playoust et al, 1965) and man (Austad et al, 1967; Meihoff and Kerr, 1968; Low-Beer et al, 1969).

Malabsorption of bile acids in the small intestine results in a greatly increased load of bile acids entering the colon where they cause secretion of water and electrolytes (Mekhjian et al, 1971) resulting in an urgent, watery diarrhoea.

In 1967 Hofmann introduced the term 'cholerheic enteropathy' to describe this type of diarrhoea and later defined two syndromes of bile acid malabsorption following ileal resection -cholerheic and steatorrhoeic diarrhoea (Hofmann, 1972). In cholerheic diarrhoea the length of ileum resected is less than 100cm, faecal fat less than 20g (30mmol) per day and faecal weight less than 900g per day. Other workers have stressed the importance of the length of any associated colonic resection since in patients with ileal resection a greater load of fluid may be delivered to the colon (Cummings et al, 1973). The occurrence of diarrhoea from other causes, such as fat malabsorption as a result of pancreatic insufficiency, may cause some malabsorption of bile acids. Meihoff and Kern in 1968 demonstrated that mannitol induced diarrhoea resulted in a two fold increase in cholic acid turnover, as a result of the diarrhoea rather than as a cause. Following ileal resection cholic acid turnover increased eleven fold.

Successful treatment of the diarrhoea following ileal resection with the bile acid sequestering agent cholestyramine

has been reported (Hofmann and Poley, 1969) but as bile acids are bound in both the small and large intestine fat malabsorption occurs with worsening of steatorrhoea and supplements of fat soluble vitamins are required.

The elemental diet Vivonex was reported to reduce faecal bile acid excretion in three healthy volunteers (Crowther et al, 1973) and the experiments with normal rats reported in previous chapters also showed a reduced turnover of bile acids during elemental diet feeding.

AIM OF STUDY

To investigate the effect of the elemental diet Vivonex on human faecal bile acid excretion, particularly in patients with cholerheic diarrhoea, and to evaulate its use in the management of these patients.

METHODS

Six patients with cholerheic diarrhoea following ileal resection, four patients with non-cholerheic diarrhoea and two volunteers were studied. The length of the ileum and colon resected (measured at surgery) together with the final diagnosis for the ten patients is shown in Table 9.1.

Two five day faecal collections were made for each subject. The first was a control collection when the subjects were consuming a normal diet containing approximately 70g of fat. Since no metabolic ward facilities were available the diet could not be strictly controlled and indeed the second normal volunteer and patient number three were studied on an out-patient basis. The second faecal collection was started three to ten days after the normal diet had been replaced by Vivonex alone. The number of stools per day was recorded on stool

charts and Vivonex intake noted daily.

The stools were weighed, homogenised and analysed in duplicate for bile acids as described in Chapter 5. The results are expressed as mg/24h. Faecal bile acid excretion was determined in eleven volunteers to establish the normal range. This was found to be 49-379 mg/24h with a mean value of 156mg/24h, and was mostly the lithocholic and deoxycholic acid peaks.

Faecal fat was determined as part of the routine screeing of the patients by a gravimetric method in the biochemistry laboratory (normal excretion less than 20mmol/24h).

RESULTS

The timing of the second faecal collection, after the normal diet was replaced by Vivonex, and the daily intake of Vivonex during the study is shown for the twelve subjects in Table 9.2. Most subjects managed to take the daily treatment of six packets of Vivonex, which supplies 1300 kilocalories, after careful introduction of the diet at half strength as recommended. Availability of beds in the ward meant that some patients were taking Vivonex for less than a week before the test faecal collection was commenced.

Tables 9.3 and 9.4 show the effect of Vivonex on faecal weight, stool frequency and the excretion of total faecal bile acids and faecal fat. Faecal wet weight was significantly reduced from 339 $\pm$ 106 (Mean $\pm$ SEM) to 146 $\pm$ 13g/24h for the six patients with cholerheic diarrhoea during treatment with Vivonex. ($p < 0.05$, Wilcoxon's signed ranks test). The small numbers and variable results for the patients with non-cholerheic diarrhoea and the normal subjects preclude statistical analysis,

but faecal wet weight during Vivonex intake was reduced in all subjects except the patients with irritable bowel syndrome (Patient 10). Faecal dry weight was reduced in all twelve subjects studied, however, and for the cholerheic diarrhoea group the dry weight was significantly reduced from 46 ± 6 (Mean ± SEM) to 13 ± 4g/24h. Stool frequency was reduced in all patients except patient 10.

Total faecal bile acids were significantly reduced in the patients with cholerheic diarrhoea from 2556 ± 664 (Mean ± SEM) to 1073 ± 445 mg/24h during treatment with Vivonex ($p < 0.05$, Wilcoxon's signed ranks test). Bile acid excretion was reduced in all six patients, the largest reduction being observed in the patient with an intact ileo-caecal sphincter (patient 5). Faecal fat excretion was also reduced during Vivonex treatment in the five patients for whom it was measured.

There was a modest increase in faecal bile acid excretion in the patient with irritable bowel syndrome (patient 10) which corresponds with the increase in wet weight of faeces and stool frequency observed. The bile acid excretion remained well within the normal range (49-379mg/24h), however, as did that of the first normal subject (number 11) whose faecal bile acid and fat excretion increased slightly during Vivonex intake. Faecal bile acids did decrease in the other four subjects in the non-cholerheic diarrhoea and normal groups during Vivonex intake.

The dramatic reduction in the diarrhoea of patients 7 and 9, assessed from the measurements of faecal wet weight, probably results from the low fat content of Vivonex. These two patients

had markedly elevated faecal fat excretion (greater than 30mmol/24h) which was probably the cause of their diarrhoea (steatorrhoeic diarrhoea). The bile acid excretion of 404mg/24h during the control period for patient 8 with a small ileal resection, is only slightly outside the normal range and was not considered sufficient to be the cause of the diarrhoea.

The excretion of individual bile acids (or groups of bile acids - see chapter 5) is shown in Table 9.5. In five of the patients with cholerheic diarrhoea most of the excess faecal bile acids during the control period were the primary bile acids cholic and chenodeoxycholic acids, as expected. Patient 2, however, excreted mainly the secondary bile acids lithocholic and deoxycholic acids. Patient 8 with the small ileal resection (in the non-cholerheic diarrhoea group) also excreted primary bile acids although the total amount was not markedly increased. The relative proportions of the four bile acid peaks showed no real change during Vivonex treatment although secondary bile acids appeared in patient 1 and the deoxycholic acid peak disappeared for patient 7. Patient 2 probably excreted a small amount of chenodeoxycholic acid during the control period but the peak was masked by the large deoxycholic acid peak and is not of quantitative importance. Measurements are near the detection limit, especially for cholic acid, in patients with excretion in the low normal range and so the results for the subjects without cholerheic diarrhoea cannot be interpreted.

DISCUSSION

The results for the six patients with cholerheic diarrhoea show that replacement of the normal diet by Vivonex for eight to fifteen days resulted in a significant reduction of faecal

bile acid excretion with a concommitant improvement in their diarrhoea, as measured by faecal wet weight and stool frequency.

Faecal wet weight was also reduced during Vivonex intake in five of the six subjects with non-cholerheic diarrhoea and normal subjects. Faecal bile acid excretion was reduced in four of these five subjects as reported by Crowther et al (1973) for three normal volunteers. The marked improvement in the diarrhoea of patients 7 and 9 who had steatorrhoea is probably a direct result of the low fat content of Vivonex reducing the amount of fatty acids and their bacterial products in the colon (Hofmann, 1972). Variable effects of a similar elemental diet (Vivasorb) on faecal wet weight were found in eighteen patients with gastro-intestinal disorders but faecal bile acids were not measured (Axelsson and Justesen, 1977).

The reduced faecal bile acid excretion during Vivonex therapy in man could result from decreased secretion of bile acids into the small intestine or increased reabsorption from the gut. In man the gallbladder can store more than 90% of the bile acid pool between meals (Wheeler, 1971) whereas in the rat, which has no gallbladder, about 93% of the total bile acid pool remains in the lumen and wall of the intestinal tract at all times (Ho, 1976). Fat and protein restricted diets have been shown to reduce the frequency of enterohepatic cycling in man, probably as a result of reduced gallbladder stimulation (Hepner, 1975), and in monkeys the daily bile salt secretion was reduced by a low fat intake (Campbell et al, 1972). A change from a solid to formula diet with the same fat content has been associated with a decrease in bile acid production (Bergström, 1962). Thus Vivonex may reduce the amount of

bile acids entering the duodenum.

Although the components of dietary fibre bind bile acids in vitro, the reported effects on bile acids in vivo, as reflected by human bile acid excretion, are conflicting (Cummings, 1973). The fat content of the diet influences the effect of fibre on faecal bile acids (Antonis and Bersohn, 1962) but reducing the crude fibre content of a low fat diet reduced faecal bile acid excretion (Antonis and Bersohn,1962) and excretion was lower on the low fat elemental diet Vivonex, than a low fat solid diet in three healthy volunteers (Crowther et al, 1975). Addition of cellulose or Metamucil to the diet increased the excretion of ^{14}C cholate in faeces (Stanley et al, 1973) as did pectin (Miettinen and Tarpila, 1977). The zero fibre nature of Vivonex may thus enhance absorption of bile acids by reducing the binding and adsorption to dietary residue in the gut.

Dietary fibre may also effect gut transit time although the reported effects are variable and seem to depend on the original transit time in each subject (Cummings, 1973: Eastwood et al, 1975; Harvey et al, 1975). A later controlled study showed that bran accelerates slow transit although it apparently slows fast transit as well (Payler et al, 1975). Perrault and co-workers (1975) found that only 2 out of 9 subjects consuming an elemental diet (Flexical) eliminated 80% of ingested markers within 96 hours whereas in their previous study 23 out of 25 subjects on a normal diet passed 80% of the markers within 96 hours. Thus Vivonex may enhance absorption of bile acids by prolonging transit time, thus increasing the time of contact for absorption, as well as reducing the binding of bile acids

to residue in the gut. In the patients with cholerheic diarrhoea following ileal resection these effects may be particularly important in enhancing bile acid absorption by the remaining ileum.

Alteration of gut flora may also affect bile acid absorption. The original reports that elemental diet feeding produced a marked reduction in faecal flora (Winitz et al, 1966 and 1970) were not confirmed, although a reduction in enterococci and increase in enterobacteria were found (Attebury et al, 1972; Crowther et al, 1973; Bounous and Devroede, 1974).

The reduction in faecal bile acid excretion, especially in the patients with cholerheic diarrhoea, that was observed in this study may result from both a reduced secretion of bile acids and enhanced reabsorption.

In patients with Crohn's disease (with and without ileal resection) elemental diet therapy for the treatment of cholerheic diarrhoea, as an alternative to Cholestyramine, has additional advantages in that it is readily assimilated and may promote healing of fistulae (Russell, 1975). The successful use of an elemental diet for long term nutritional support in Crohn's disease has been reported (Goode et al, 1976).

Group	Patient No.	Length resected (cm)		Diagnosis
		Ileum	Colon	
Cholerheic Diarrhoea	1	25	22	Carcinoma of caecum
	2	7	6	Mesenteric thrombosis
	3	40	30	Crohn's disease
	4	35	40	Crohn's disease
	5	90*	0	Crohn's disease
	6	100	50	Crohn's disease
Non-cholerheic Diarrhoea	7	0*	0	Chronic pancreatitis
	8	10	0	Villous papilloma
	9	0*	0	Crohn's disease
	10	0*	0	Irritable bowel syndrome

TABLE 9.1

PATIENTS IN WHOM THE EFFECT OF VIVOTEX ON FAECAL BILE ACIDS WAS STUDIED.

* ileocaecal sphincter intact

Patient Group and number		Days of Vivonex intake before faecal collection started	Daily intake (packets/24h)
Cholerheic diarrhoea	1	3	6
	2	7	6
	3	7	6
	4	7	3-4
	5	7	4
	6	8	4
Non-cholerheic diarrhoea	7	5	6
	8	7	6
	9	9	6
	10	3	6
Normal subjects	11	6	6
	12	8	4

TABLE 9.2

VIVONEX INTAKE AND TIMING OF TEST COLLECTION

Patient Group and Number		Wet Weight of Faeces (g/24h) C	Wet Weight of Faeces (g/24h) V	Dry Weight of Faeces (g/24h) C	Dry Weight of Faeces (g/24h) V	Stool Frequency (number/24h) C	Stool Frequency (number/24h) V
Cholerheic Diarrhoea	1	250	148	42	35	2-5	0-4
	2	200	186	41	17	4-6	3-5
	3	171	126	29	10	6-8	3-5
	4	853	176	66	13	6-10	5-7
	5	360	134	59	16	1-4	0-3
	6	199	105	39	18	3-4	2-3
	Mean ±SEM	339* ±106	146 ±13	46 * ±6	18 ±4		
Non-Cholerheic Diarrhoea	7	780	150	81	3	3-6	0-4
	8	176	75	25	9	6-8	1-3
	9	425	101	86	6	1-5	1-3
	10	205	245	43	18	0-6	1-10
Normal Subjects	11	58	35	13	7	1	0-1
	12	89	28	22	9	1	0-1

C = control collection
V = Vivonex intake

* control v Vivonex
$p < 0.05$ (Wilcoxon's signed ranks test)

TABLE 9.3 EFFECT OF VIVONEX INTAKE ON FAECAL WEIGHT AND STOOL FREQUENCY

Patient Group and Number		Total Faecal Bile Acids (mg/24h) C		Faecal Fat (mmol/24h) C	V
Cholerheic Diarrhoea	1	4903	3229	29.7	-
	2	860	657	15.1	5.0
	3	1638	986	11.4	0.4
	4	1473	189	62.9	6.6
	5	4212	824	31.0	19.0
	6	2247	552	33.9	11.0
Mean ±SEM		2556 ±664 *	1073 ±445		
Non-Cholerheic Diarrhoea	7	184	16	121.3	-
	8	404	271	12.8	4.0
	9	249	28	89.2	33.6
	10	105	117	15.7	-
Normal Subjects	11	80	260	2.4	4.4
	12	168	49	3.0	15.2

C = control collection
V = during Vivonex intake

* Control v Vivonex
$p < 0.05$ (Wilcoxon's signed ranks test)

TABLE 9.4 EFFECT OF VIVONEX INTAKE ON TOTAL FAECAL BILE ACIDS AND FAECAL FAT EXCRETION

Patient Group and Number		Bile Acid Excreted (mg/24h)							
		Control				Vivonex			
		LC	DC	CDC	C	LC	DC	CDC	C
Cholerheic Diarrhoea	1	0	0	1641	3262	462	647	758	1362
	2	304	487	0	69	217	412	28	0
	3	179	233	436	790	17	91	228	651
	4	0	0	582	891	0	0	109	79
	5	0	0	1694	2518	0	0	448	377
	6	0	0	676	1571	0	0	278	274
Non-Cholerheic Diarrhoea	7	0	129	54	0	0	1	3	12
	8	0	0	194	211	0	0	119	153
	9	46	203	0	0	9	19	0	0
	10	28	57	21	0	21	49	26	21
Normal Subjects	11	17	27	24	13	182	72	6	0
	12	51	79	37	0	13	25	11	0

TABLE 9.5 FAECAL EXCRETION OF INDIVIDUAL BILE ACIDS DURING CONTROL PERIOD AND VIVONEX INTAKE

CHAPTER 10

COMPARISON OF VIVONEX AND A STANDARD TEST MEAL ON HUMAN PANCREATIC SECRETION

INTRODUCTION

Clinical findings suggest that elemental diets may significantly reduce exocrine pancreatic stimulation. Voitk and co-workers (1973) reported that five patients with complicated pancreatitis, unsuitable for intravenous feeding and unable to tolerate normal food, tolerated the elemental diet 'Flexical' well, with spontaneous closure of pancreatic fistulae and complete recovery in one case. Similarly closure of pancreatic fistulae was reported with an oral elemental diet (McArdle et al, 1972).

Studies in man (Bury and Jambunathan, 1974) and dogs (McArdle et al, 1972) have shown a reduction of gastric secretion by elemental diets and the latter study and that of Brown et al (1970) reported a reduction in trypsinogen levels of the pancreas in dogs. Oral and duodenal administration of an elemental diet in dogs produced significant pancreatic secretion compared with intravenous hyperalimentation although less than with exogenous cholecystokinin (Kelly and Mahrwold, 1976) and in another study an oral elemental diet produced a strong pancreozymin type response in dogs (Ragins et al, 1973).

The effect of oral elemental diets in man on pancreatic secretion has not been fully investigated, but early clinical findings (McArdle et al, 1972; Voitk et al, 1973) suggest that a marked reduction compared with normal food occurs, which contrasts with the experimental results in dogs (Ragins et al, 1973; Kelly and Mahrwold, 1976). Duodenal and jejunal amino acid perfusion in man (Go et al, 1970; Ertran et al, 1971), was shown to stimulate exocrine pancreatic secretion.

AIMS OF STUDY

To investigate the effect of Vivonex on pancreatic function by measuring the tryptic activity of duodenal contents after oral ingestion of Vivonex and a Lundh test meal in the same subjects.

This is important for three reasons:

1. To predict the possible value of Vivonex in treatment in acute pancreatitis.
2. Altered pancreozymin stimulation also affects gallbladder function and hence bile acid metabolism.
3. Altered pancreatic and biliary secretion may affect small bowel structure and function.

METHODS

Design of Study

Five normal volunteers (3 male, 2 female) aged 17-24 years were studied. After an overnight fast, a radio-opaque (5 mm O.D., 3 mm I.D.) tube with collection holes in the terminal 5 cm was passed orally and the collection point positioned in the third part of the duodenum under radiographic control. The tube position was checked again at the end of the collection period to ensure that it had not moved back, especially into the stomach. Once the tube was correctly positioned gentle suction was applied using a Watson-Marlow pump and basal duodenal juice collected for 15 minutes. The subjects then drank either the Lundh test meal or Vivonex (Table 10.1) and the duodenal juice was collected for four consecutive half-hour periods into ice. The volume collected was noted and the samples either frozen at -20°C or assayed for trypsin activity immediately. Each subject was studied twice, once for each meal, in random order.

Measurement of Trypsin Activity

The method used was that described by Wiggins (1967).

The rate at which H^+ is liberated by the hydrolysis of the synthetic substrate N benzoyl-L-arginine ethyl ester (B.A.E.E.) is measured by finding the time taken to neutralise a known amount of alkali. The conditions of the assay are set so that the pH stays within the range 7.9-8.4, where changes in enzyme activity with pH are small.

The substrate solution was prepared by dissolving 0.5g of B.A.E.E. in 10ml of 1% sodium barbiturate, adjusting to pH9 and bringing the volume to 100ml with distilled water. For each assay, 10ml of substrate and 1.8ml acetate buffer (0.05M pH 5.8, containing 0.5g of $CaCl_2$ per litre) were mixed and brought to 25°C in a water bath, on a magnetic stirrer with the pH electrode immersed. An aliquot (0.2ml) of duodenal sample was then added bringing the pH to about 8.5, falling continuously. When the pH was reading 8, sodium hydroxide (0.2ml of 0.04N Na OH) was added and a stopclock started. The pH was followed until it again reached 8 and the stopclock was then stopped. The time shown was that during which 8 μeq H^+ had been released.

If the time interval was greater than 10 minutes the assay was repeated using 0.4ml of sample and 1.6ml of buffer. If the reaction still took longer than 10 minutes the result was recorded as less than 2μeq ml^{-1} min^{-1}.

Results were calculated as follows:

Trypsin activity (μeq H^+ released per min per ml of intestinal contents)

$$= \frac{\text{meq NaOH added}}{\text{time (min)}} \times \frac{1}{\text{vol of intestinal contents assayed}}$$

$$= \frac{3}{\text{time}} \times \frac{1}{\text{vol assayed}}$$

All samples were assayed in duplicate and the mean tryptic activity for the first and second hours, together with the mean for the entire two hour period, was calculated.

The correlation coefficient for duplicate assays (21 samples) was 0.932 and the coefficient of variation was 7.6% (see chapter 5 for calculation). Differences between results for the two meals for each subject were compared using a paired t-test.

RESULTS

The volume of duodenal juice collected during the first hour following ingestion of the Lundh test meal was 67 ± 43ml (Mean ± SEM and after Vivonex 25 ± 11ml. During the second hour, the volume collected after the Lundh meal was 48 ± 12 ml and for Vivonex 23.2 ± 14 ml. The volume collected probably reflects the volume of the duodenal contents, although in the absence of non-absorbable dilution marker (eg. polyethylene glycol, 4000) no reliable estimate can really be made.

The mean tryptic activity for the five subjects following the Lundh and Vivonex meals is shown for the two hour period (Figure 10.1) and the first and second hours separately (Figures 10.2 and 10.3). Mean tryptic activity over the two hour period was reduced by Vivonex compared with the Lundh test meal in four of the five subjects but the difference was not statistically significant. Similarly, for three of the five subjects, mean tryptic activity during the first hour

following Vivonex was less than following the Lundh meal.

Mean tryptic activity during the second hour was less following Vivonex than the Lundh meal in all five subjects and the difference between the two meals is statistically significant ($p < 0.025$).

DISCUSSION

The results of this study show that significant pancreatic stimulation and secretion of trypsin occurs following oral ingestion of the elemental diet Vivonex in normal healthy subjects. The elemental diet does, however, seem to have a less prolonged effect or a less marked effect than the Lundh meal used in standard pancreatic function tests (Cook et al, 1967), since the tryptic activity of duodenal contents was significantly less, during the second hour, following Vivonex. The reduced tryptic activity is unlikely to be due to dilution of duodenal contents since the volume collected after Vivonex was less than after the Lundh meal.

These results are in agreement with a recently published study in human subjects (Vidon et al, 1973) where lower chymotrypsin secretion was produced by continuous jejunal perfusion of Vivonex than by an equicaloric infusion of crushed food homogenate. These workers concluded that the stimulative effect on the pancreas could be directly related to the nitrogen load. The reduced tryptic activity during the second hour of this study may therefore be due to the lower nitrogen content of the Vivonex compared with the Lundh test meal.

Jejunal and duodenal perfusion with amino acids has been shown to stimulate pancreatic secretion of enzymes to the same extent

as endogenous cholecystokinin-pancreozymin (Go et al, 1970; Ertran et al, 1971) but intragastric administration of amino acids in a patient with biliary and pancreatic fistula resulted in no stimulation of pancreatic secretion (Ragins et al, 1973) and five out of six patients with complicated pancreatitis were successfully treated with the elemental diet Flexical intragastrically (Voitk et al, 1973). Intrajejunal glucose inhibits pancreatic secretion in man (Dyck, 1971) and the high glucose content of Vivonex may also be a factor in the reduced tryptic activity found in this study.

Thus although amino acids are potent stimulators of pancreatic secretion when infused into the upper small intestine in man, intragastric administration of amino acids alone, or as components of an elemental diet as in this study, results in a less marked stimulation of pancreatic secretion.

The reduced stimulation of trypsin secretion by Vivonex observed in the study indicates a reduced cholecystokinin-pancreozymin response (Go et al, 1970), which would also reduce gallbladder stimulation, as suggested by the results of Vivonex feeding on faecal bile acid excretion in man (Chapter 3). A reduction in pancreatic and biliary secretion may also affect small bowel structure and function (Altmann, 1971; Weser et al, 1977; Eckmauer et al, 1973).

Since significant stimulation of trypsin secretion did occur with Vivonex it does not seem to offer a viable alternative to intravenous feeding for the treatment of an acute attack of pancreatitis, although it may be helpful in patients unsuitable for intravenous nutrition.

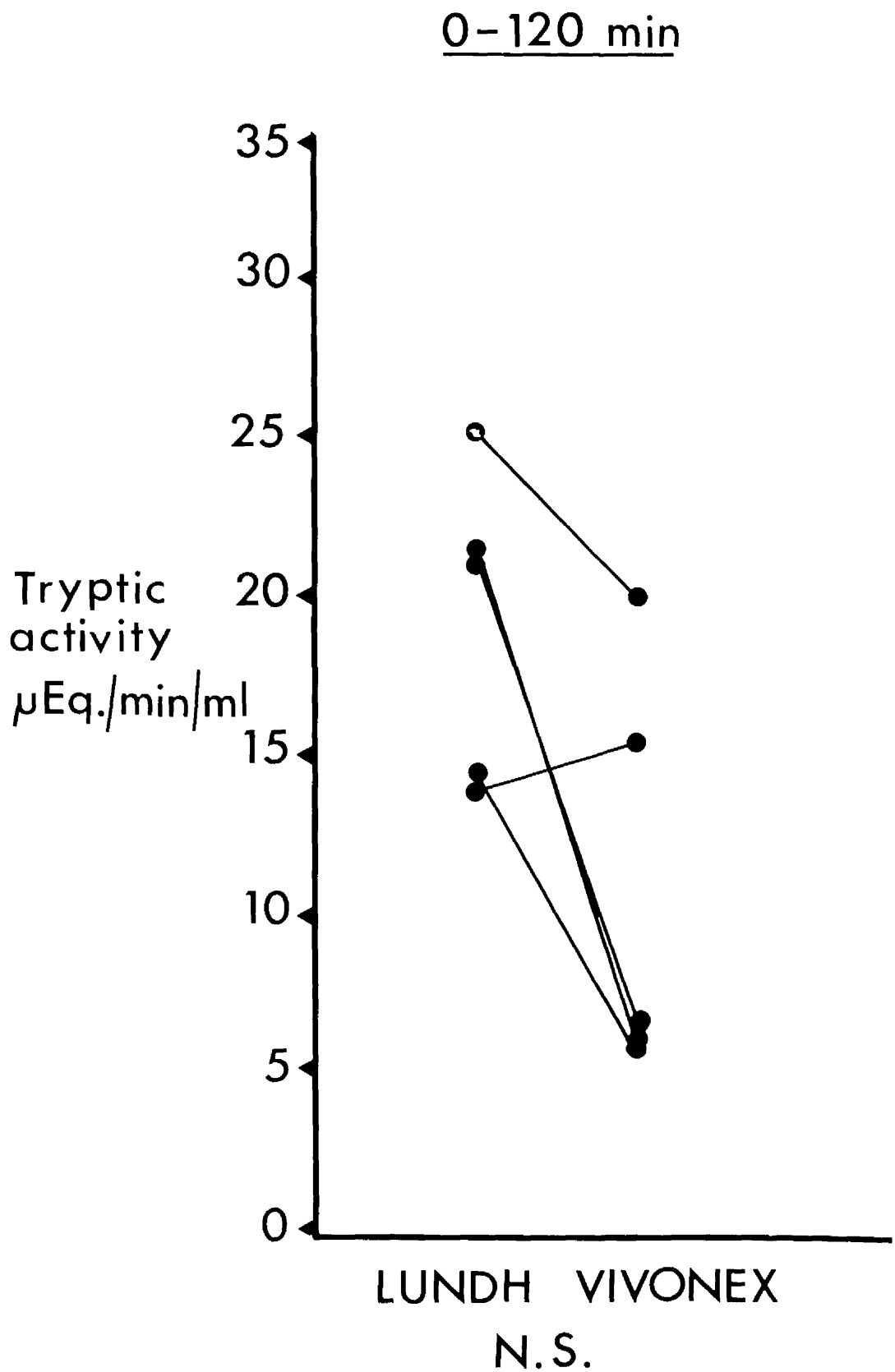


FIGURE 10.1. TRYPTIC ACTIVITY FOR THE FIVE SUBJECTS FOLLOWING INGESTION OF THE LUNDH TEST MEAL AND VIVONEX. MEAN RESULTS FOR THE TWO HOUR COLLECTION PERIOD

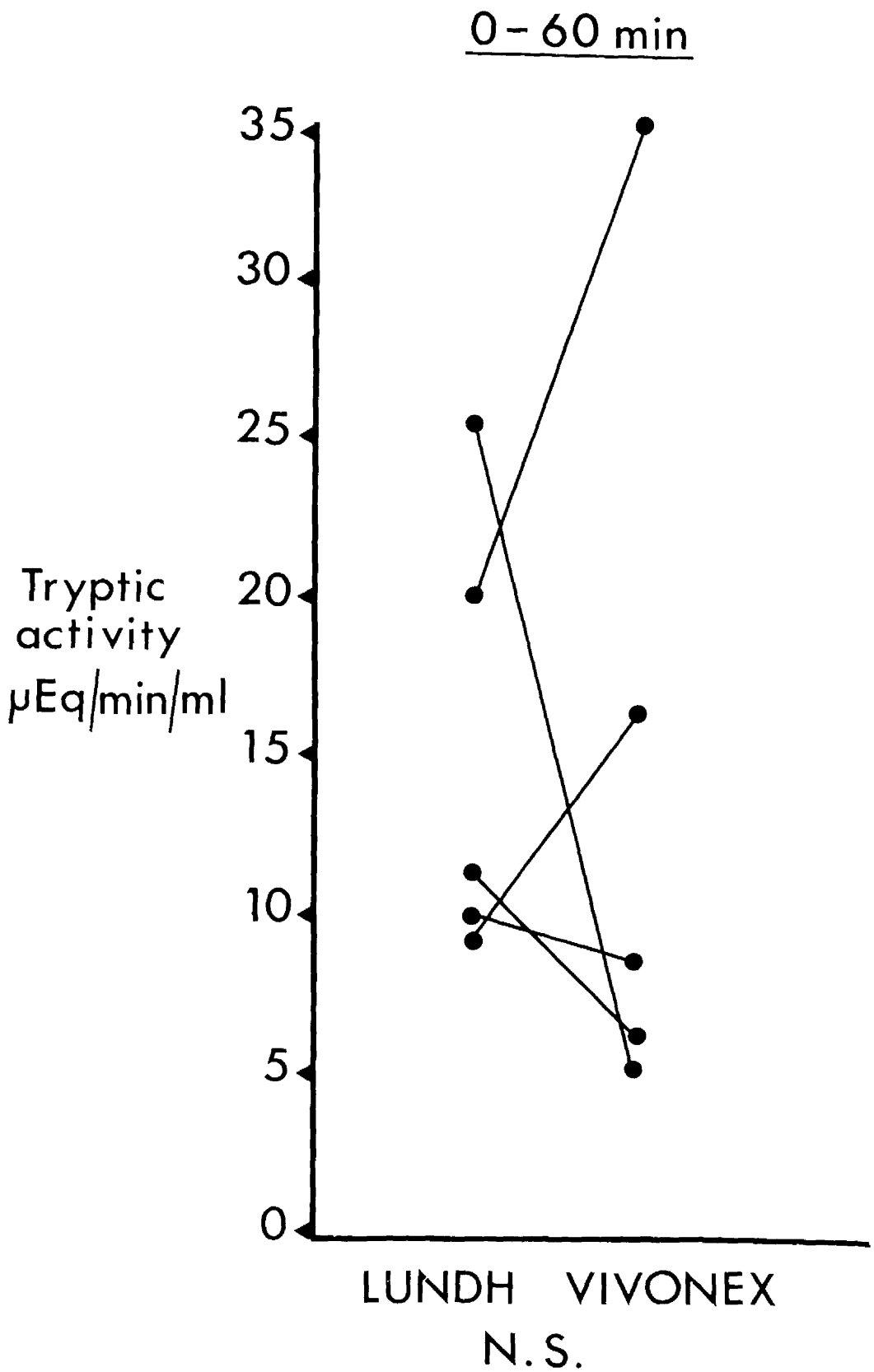


FIGURE 10.2. TRYPTIC ACTIVITY FOR THE FIVE SUBJECTS IN THE FIRST HOUR FOLLOWING INGESTION OF THE LUNDH TEST MEAL AND VIVONEX

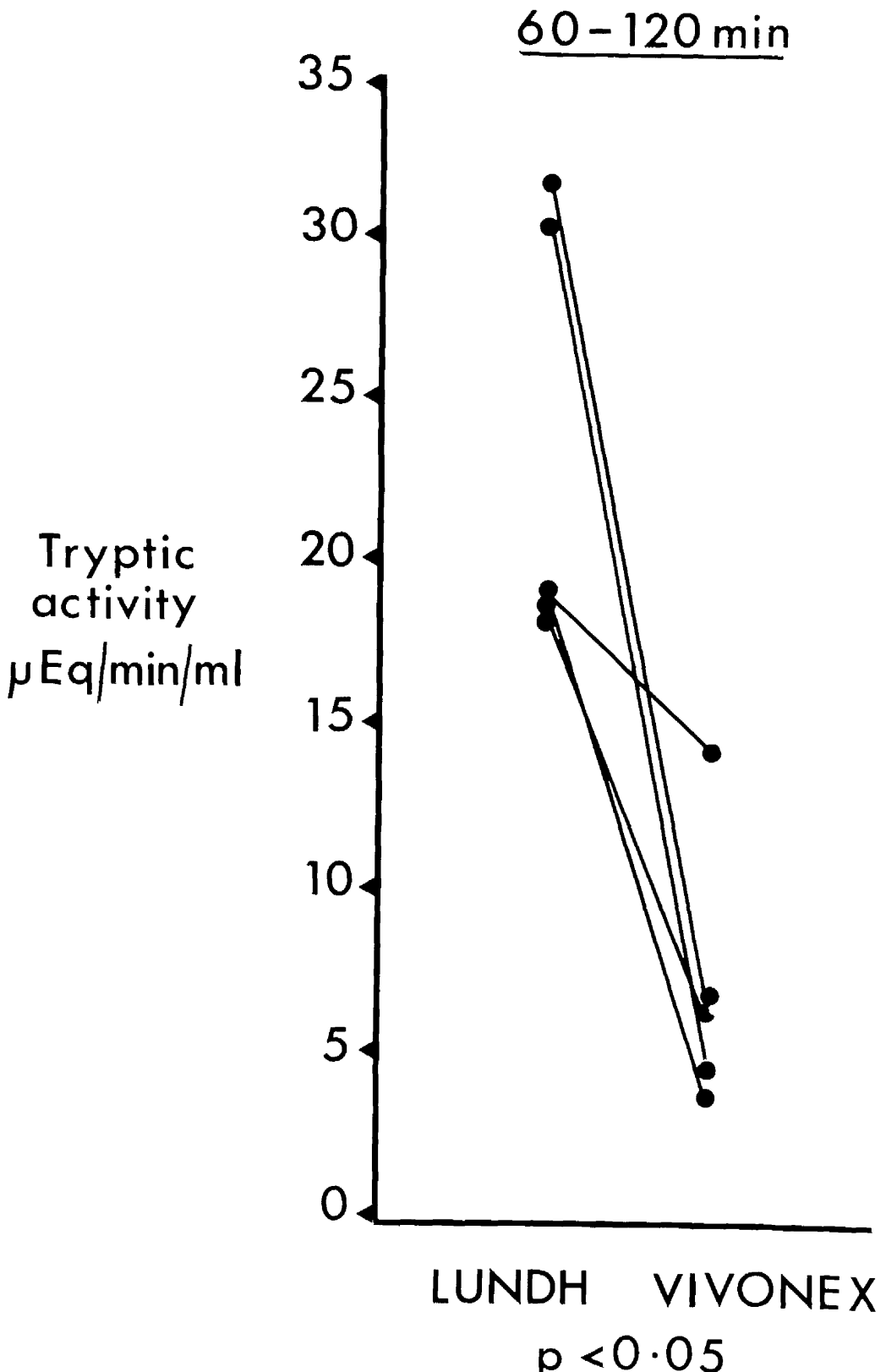


FIGURE 10.3. TRYPTIC ACTIVITY FOR THE FIVE SUBJECTS IN THE SECOND HOUR FOLLOWING INGESTION OF THE LUNDH TEST MEAL AND VIVONEX

Lundh Meal	13g Soya bean oil 15g Casilan (dried milk powder) 40g Glucose Supplies - 2.4g nitrogen 250 calories in 300ml
Vivonex Meal	0.435g Safflower oil 6.34 g L-amino acids 69g Glucose Supplies - 0.98g nitrogen 300 calories in 300ml

TABLE 10.1

COMPOSITION OF LUNDH AND VIVONEX TEST MEALS

CHAPTER II

THE EFFECT OF VIVONEX AND FLEXICAL ON THE MORPHOLOGY OF RAT JEJUNUM AND ILEUM

INTRODUCTION

The importance of oral rather than intravenous nutrition in maintaining normal gut mass and function has been demonstrated in rats (Levine et al, 1974; Johnson et al, 1975) and dogs (Feldman et al, 1976). Villus height and crypt height were measured by Levine et al (1974) and found to be reduced in the jejunum of intravenously fed rats but not in the ileum. Bypassed jejunum in orally fed rats showed reduced villus height and decreased absorption of glucose (Gleeson et al, 1972) and these changes were also attributed to lack of luminal nutrition. Following jejunal resection, villus height and absorptive function of the residual rat ileum increase, and the increased nutrient load reaching the ileum has been implicated in these adaptive changes (Dowling and Booth, 1967). Similar changes were also found when the jejunum and ileum were transposed (Dowling and Booth, 1967). The necessity of oral or luminal nutrition for the adaptive changes following partial small bowel resection has also been demonstrated in the dog, using intravenously fed controls (Feldman et al, 1976).

The effect of different types of oral nutrition, and in particular the influence of elemental diets, on small bowel structure and function has not been fully investigated. Elemental diets were shown to be beneficial during the adaptive phase of short gut syndrome in man (Voitk et al, 1973) and have been used for nutritional support in Crohn's disease (Goode et al, 1976) but these are essentially uncontrolled clinical studies. Animal studies have shown that rats recovered their normal appetite much faster after abdominal irradiation when fed an elemental diet (Pareau, 1975), and a diet containing hydrolysed

casein promoted faster recovery of the intestinal mucosa in irradiated mice than whole casein or normal rat chow (Huggon, 1976). Work published in abstract form has shown, in the dog, that perfusion of Thiry-Vella loops with Vivonex results in increased villus height compared with saline perfused and uninfused loops (Jacobs et al, 1975).

AIM OF STUDY

1. To investigate the effect of feeding the elemental diet Vivonex on the morphology of normal rat jejunum.
2. To compare the effects of Vivonex and Flexical feeding for one month on the morphology of rat jejunum and ileum.

EXPERIMENTAL

Groups Studied

Part 1 Three groups of twelve rats were studied for three months with equal numbers in each group fed Vivonex and normal rat diet (Oxoid 41B). These rats (Groups 2,3 and 4) were housed in metabolic cages and also used for studies of faecal bile acid excretion (Chapter 6). A further group of rats was studied for one month with four fed Vivonex and four the control diet (Group 6).

Jejunal morphology was examined in these rats on 5cm segments of small intestine taken 30 cm distal to the pylorus.

Part 2 Two groups of eighteen rats (Groups 7 and 8) with equal numbers fed Vivonex, Flexical and control diet, were studied for a period of one month. These rats were housed in normal cages with wide mesh bottoms. Jejunal and ileal morphology was examined after perfusion of the jejunum (Chapter 15). The segment of jejunum was taken from the

mid point of the perfused segment (25-30cm from pylorus) and ileum was taken 50cm proximal to the ileo-caecal valve

Methods

The segments of small intestine (5cm) were immediately fixed in formol-saline, cut longtitudinally in half and embedded in paraffin. Sections 5-7μ thick were cut giving four strips per section, and stained with standard Haematoxylin and Eosin. Villus height and crypt height were measured using a calibrated Leitz micrometer eyepiece (x10) and a total magnification of x100. Only well orientated intact villi and crypts perpendicular to the lamina muscularis were measured. Between 12 and 20 measurements were made for each section, usually 15-16 villi being measured, and the results expressed in μm. The crypt height was divided by the villus height to give the ratio.

Dr. F. D. Lee, Consultant in the Department of Pathology at Glasgow Royal Infirmary, kindly arranged preparation of the specimens of small intestine in his department. The sections were examined qualitatively by myself and as a result of the obvious visual changes Dr Lee kindly performed the measurements of villus and crypt height.

Statistics

The significance of differences between control and elemental diet fed rats was analysed using Wilcoxon's Sum of Ranks test and comparisons of the jejunum and ileum in the same rats analysed using Wilcoxon's Signed Ranks test (Langley, 1968).

RESULTS

The weight gain of the rats in Groups 2-4 and 7 and 8 has been detailed elsewhere (Chapters 6 and 15). Vivonex fed rats

in some groups showed an initial reduction in weight gain but by the end of the studies, body weight of the elemental diet fed rats was either not significantly different from the controls or, in some cases, was in fact greater than the controls. In no instance was the weight of the controls significantly greater than that of the rats fed an elemental diet. The results for group 6 rats showed the same pattern with no significant difference between the final weight of controls and Vivonex fed rats at the end of the month. Mean ($\pm$ SEM) weight for the controls was 278 $\pm$ 12g and 262 $\pm$ 7g for the rats fed Vivonex.

Part 1

The results of Vivonex feeding for three months on jejunal villus height (VH), crypt height (CH) and the cryot height: villus height (CH:VH) ratio for groups 2,3 and 4 are shown in Tables 11.1, 11.2 and 11.3. The ratio of CH to VH was significantly reduced in the jejunum of the rats fed Vivonex compared with controls in each group ($P < 0.01$ in each case). Villus height was significantly increased for the Vivonex fed rats in each group ($p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively) but the reductions in crypt height were not significant. When the results for the three groups were combined (Table 11.4) the reduction in crypt height was just significant ($p < 0.05$) and the increased villus height and decreased CH:VH ratio were highly significant ($p < 0.002$ in each case).

The results for Group 5 (Table 11.5) demonstrate that these differences occur after one month of Vivonex feeding. The CH:VH ratio was significantly reduced from 0.25 $\pm$ 0.02 μm in the controls to 0.17 $\pm$ 0.00 μm in the rats fed Vivonex ($p < 0.05$). The histological appearance of the jejunum of a control and Vivonex

fed rat are shown in Figure 11.1.

Part 2

The results for the effects of one month of Vivonex and Flexical feeding on jejunal morphology are shown in Table 11.6 (Group 7) and Table 11.7 (Group 8). There were no significant differences between the rats fed Vivonex and those fed Flexical. The CH:VH ratio was significantly reduced in the Vivonex and Flexical fed rats compared with controls in Group 7 ($p < 0.01$, $p < 0.05$) and Group 8 ($p < 0.01$, $p < 0.01$). Villus height was significantly increased from 368 ± 9 μm (Mean ± SEM) for the controls of group 7 to 465 ± 21 μm for the rats fed Vivonex ($p < 0.01$) and 473 ± 18 μm for those fed Flexical ($p < 0.01$) but in Group 8 the increase in villus height was not significant. The results (Mean ± SEM) for the two groups combined are shown in Table 11.8. Jejunal villus height was significantly increased from 394 ± 23 μm in the controls to 506 ± 21 μm in the rats fed Vivonex and 495 ± 19 μm in the rats fed Flexical ($p < 0.002$ in each case). The CH:VH ratio was reduced from 0.31 ± 0.02 in the controls to 0.22 ± 0.01 in the rats fed Vivonex and Flexical ($p < 0.002$ in each case). The reduction in CH was not statistically significant, but was the same for the two elemental diets.

The results for ileal morphology are shown in Table 11.9 for group 7 and Table 11.10 for group 8 rats. The changes observed in the ileum for the elemental diet fed rats are similar to those for the jejunum and again there were no significant differences between the two elemental diets. The CH:VH ratio was reduced in the Vivonex and Flexical fed rats compared with controls in both groups 7 and 8, although the reduction for the Vivonex fed rats in group 7 was not statistically significant. The results

for the two groups are combined in Table 11.11 and it can be seen that the changes induced in ileal morphology by elemental diet feeding are similar to those for jejunal morphology (Table 11.3). The CH:VH ratio was significantly reduced in the ileum of the rats fed Vivonex and Flexical ($p < 0.002$) as a result of a significant increase in villus height ($p < 0.002$) and decrease in crypt height ($p < 0.05$) compared with the controls.

When the morphology of the jejunum and ileum of individual rats was compared (Wilcoxons Signed Ranks test), for each of the three parameters (VH, CH and CH:VH), no significant difference in villus height between the ileum and jejunum was found for any of the diets (Table 11.12). However, crypt height and the CH:VH ratio were significantly greater in the ileum than jejunum of control and Flexical fed rats ($p < 0.05$ in all cases), but not significantly different for the rats fed Vivonex.

DISCUSSION

The results for Part 1 of this study demonstrate that Vivonex feeding produces a marked reduction in the CH:VH ratio of rat jejunum, mainly as a result of increased villus height but with reduced crypt height also contributing. In Part 2 it was demonstrated that Flexical produces the same results as Vivonex after one month of feeding. Shorter feeding periods, have not been investigated since two or three weeks seem to be required for the rats to become adjusted to the elemental diets and to gain weight satisfactorily.

No cell kinetic studies have been carried out in this study, but the marked reduction in CH:VH ratio suggests improved survival of the mature enterocyte population as result of elemental diet feeding. The functional villus cell population may control the

regulation of cell proliferation and maturation in the crypt by a feedback mechanism (Galjaard et al, 1972; Rijke et al, 1976). Changes in CH:VH ratio similar to these in the rats fed elemental diets were found in germ-free rats and 3H thymidine labelling showed an increase in villus transit time in germ free animals (Galjaard et al, 1972). Similar results have been reported for germ-free mice (Abrams et al, 1965). Thus cell turnover may be reduced in the elemental diet fed rats, and this may be due to an alteration of bacterial flora.

Hypoplasia with reduced villus height in bypassed loops of dog jejunum was prevented by saline perfusion and Vivonex perfusion was found to increase villus height (Jacobs et al, 1975). No measurements of crypt height were reported in this abstract and it may have been a hyperplastic response with increased crypt height as well, rather than an alteration of CH:VH ratio. The increased villus height is in agreement with the results of this study, however. Two groups of workers have recently published work in abstract form showing a reduced villus height and small bowel mass in rats fed Vivonex but these were short term studies of 8 days and two weeks and the rats fed the elemental diets had not gained weight as well as the controls (Dohnauer et al, 1973 b; Young et al, 1973), which may account for the different results. The CH:VH ratio was reduced in the Vivonex fed rats in the first of these studies, however.

Factors other than alteration of bacterial flora which may result in alteration of the CH:VH ratio during elemental diet feeding include reduced physical stress with a zero residue diet, enhanced luminal nutrition, alteration of pancreatic and biliary secretions and changes in the hormonal or neurovascular

regulation of gut structure and function.

Bile and pancreatic secretions have been shown to stimulate mucosal growth in rat ileum (Altmann, 1971; Weser et al, 1977) and intravenous cholecystokinin plus secretin prevented the mucosal hypoplasia of intravenous nutrition in dogs (Hughes et al, 1973). However, it is probable that if biliary and pancreatic secretion is altered during elemental diet feeding then it is a reduction rather than an increase that occurs (Chapters 9 and 10). Indirect effects of intraluminal nutrition in maintaining gut mass have been demonstrated by comparing bypassed intestine in orally and intravenously fed rats (Dworkin et al, 1976; Adams et al, 1978) and unidentified hormonal and neurovascular factors implicated. These factors may be altered by elemental diet feeding. Direct effects of dextrose and amino acid infusion on gut mass have been demonstrated in intravenously nourished intact rats (Spector et al, 1977) and it is possible that the composition of the elemental diets enhance these direct effects of oral nutrition.

The finding that the JH to VH ratio is reduced in the ileum as well as the jejunum during elemental diet feeding favours a hormonal or neurovascular mechanism, since direct effects of luminal nutrition or pancreatic and biliary secretions would not be expected to influence the distal small bowel. Lack of residue in the elemental diets resulting in reduced physical stress, or in fact an alteration of bacterial flora, could also account for the ileal changes in addition to those in the jejunum.

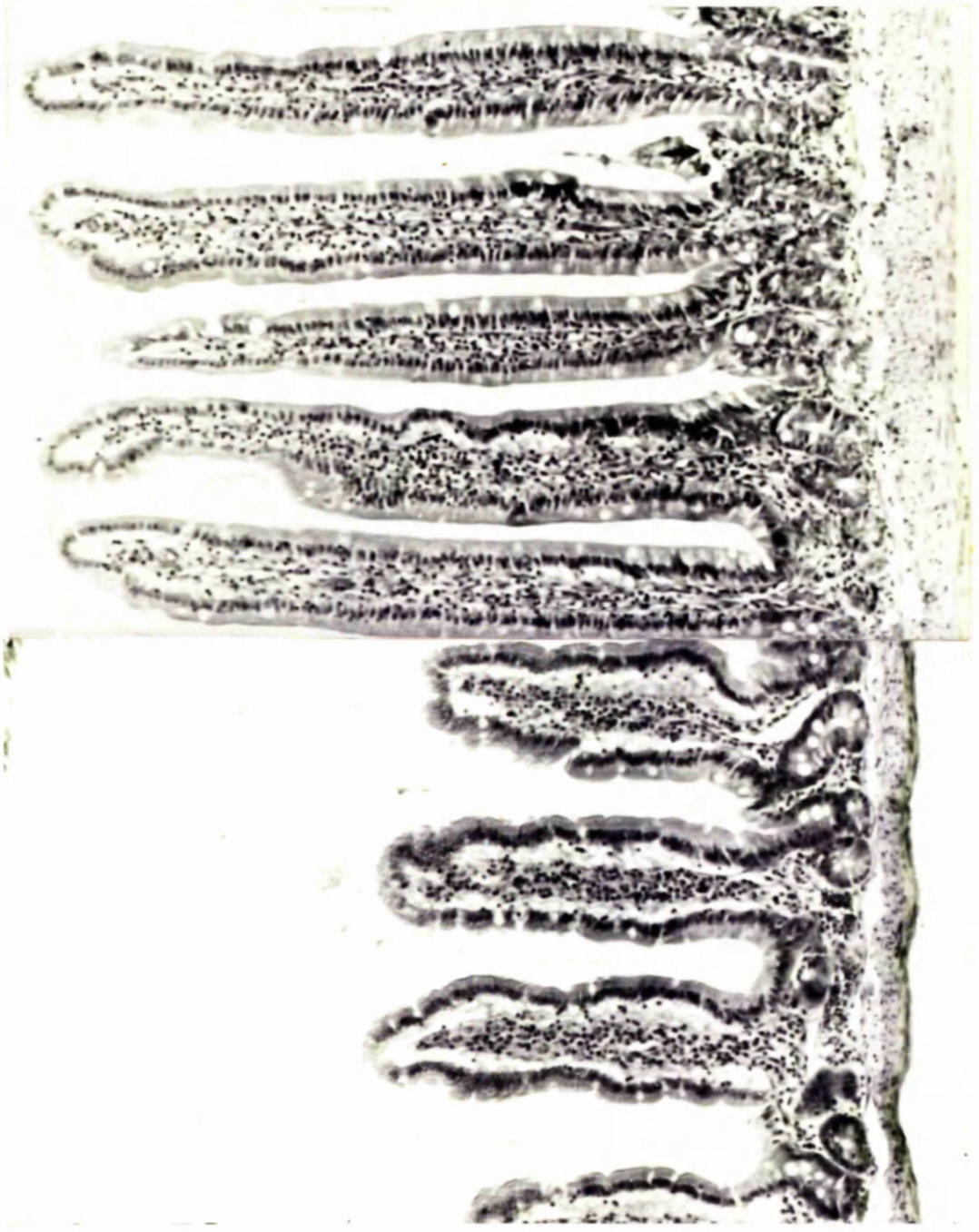
The effects of Vivonex on the ileum are greater than those of Flexical as reflected by the lack of a significant increase

in ileal crypt height, compared with the jejunum, of the rats fed Vivonex. This difference between the two diets could be mediated by different alteration of hormonal or neurovascular factors or of the bacterial flora.

The relative importance of these different factors, which include altered hormonal or neurovascular regulation, reduced physical stress with low residue diets, alteration of bacterial flora and possible direct effects of luminal nutrition, cannot be assessed from the results of this study but elemental diet feeding apparently improves enterocyte survival throughout the small intestine.

FIGURE 11.1 HISTOLOGICAL APPEARANCE OF THE JEJUNUM OF A CONTROL AND VIVONEX FED RAT

Longtitudinal sections of jejunum stained with Haematoxylin and Eosin.
Control rat on left, Vivonex-fed on right.
(photographed at the same magnification)



	Villus Height (μm)		Crypt Height (μm)		Crypt Height:Villus Height	
	Control	Vivonex	Control	Vivonex	Control	Vivonex
	679	714	145	124	0.21	0.17
	577	653	146	130	0.25	0.20
	336	594	129	120	0.39	0.20
	423	592	129	73	0.30	0.12
	581	614	194	120	0.33	0.19
	494	626	183	99	0.37	0.15
Mean ±SEM	515 * ±50	632 ±19	154 ±11	111 ± 9	0.31 ** ±0.03	0.17 ±0.01

* $p < 0.05$
** $p < 0.01$

Significance of difference between control and Vivonex fed rats

TABLE 11.1 GROUP 2 RATS: EFFECT OF VIVONEX FEEDING FOR 3 MONTHS ON THE MORPHOLOGY ON RAT JEJUNUM

Villus Height (μm)		Crypt Height (μm)		Crypt Height:Villus Height	
Control	Vivonex	Control	Vivonex	Control	Vivonex
395	541	113	133	0.28	0.28
442	719	126	129	0.28	0.17
473	836	109	136	0.23	0.16
527	600	120	125	0.22	0.20
547	642	147	117	0.26	0.18
426	561	118	82	0.27	0.14
Mean 468 **	672	122	118	0.26 **	0.17
±SEM ±24	±49	± 5	±10	±0.01	±0.01

** $p < 0.01$ for control v Vivonex fed rats

TABLE 11.2 GROUP 3 RATS: EFFECT OF VIVONEX FEEDING FOR 3 MONTHS ON THE MORPHOLOGY OF RAT JEJUNUM

	Villus Height (μm)		Crypt Height (μm)		Crypt Height:Villus Height	
	Control	Vivonex	Control	Vivonex	Control	Vivonex
	409	556	108	120	0.26	0.22
	428	521	127	95	0.30	0.18
	400	852	130	142	0.33	0.17
	557	561	124	114	0.22	0.20
	590	635	169	119	0.29	0.18
	447	635	122	109	0.27	0.17
Mean ±SEM	472 * ±33	627 ±49	130 ± 8	117 ± 6	0.28 ** ±0.02	0.19 ±0.01

* $p < 0.05$
** $p < 0.01$ for Control v Vivonex fed rats

TABLE 11.3 GROUP 4 RATS: EFFECT OF VIVONEX FEEDING FOR 3 MONTHS ON THE MORPHOLOGY OF RAT JEJUNUM

	Control (n=18)	Vivonex (n=18)	
Villus Height (μm)	435 ± 21	636 ± 22	$p < 0.002$
Crypt Height (μm)	136 ± 6	116 ± 4	$p < 0.05$
Crypt Height / Villus Height	0.28 ±0.01	0.18 ±0.01	$p < 0.002$

TABLE 11.4

EFFECT OF VIVONEX FEEDING FOR 3 MONTHS ON THE MORPHOLOGY OF RAT JEJUNUM. (COMBINED RESULTS FOR GROUPS 2, 3 AND 4)

Values are Mean ± SEM

	Villus Height (μm)		Crypt Height (μm)		Crypt Height:Villus Height	
	Control	Vivonex	Control	Vivonex	Control	Vivonex
	460	676	122	118	0.26	0.17
	599	695	130	122	0.21	0.17
	643	636	143	107	0.22	0.16
	579	707	183	126	0.31	0.17
Mean ±SEM	570 ±39	679 ±16	145 ±14	118 ± 4	0.25 * ±0.02	0.17 ±0.00

* $p < 0.05$ for control v Vivonex fed rats

TABLE 11.5 GROUP 6 RATS: EFFECT OF VIVONEX FEEDING FOR ONE MONTH ON THE MORPHOLOGY OF RAT JEJUNUM

	Control			Vivonex			Flexical		
	VH	CH	CH/VH	VH	CH	CH/VH	VH	CH	CH/VH
	353	101	0.28	409	88	0.22	494	126	0.26
	343	112	0.33	429	112	0.26	483	115	0.24
	360	119	0.33	484	125	0.26	485	116	0.24
	404	143	0.35	431	114	0.26	493	106	0.22
	371	126	0.34	547	147	0.27	383	125	0.33
	376	114	0.30	491	121	0.25	500	106	0.21
Mean ± SEM	368 ± 9	119 ± 6	0.32 ± 0.01	465 ± 21**	118 ± 8	0.25 ± 0.01**	473 ± 18**	116 ± 4	0.25 ± 0.02*

* $p < 0.05$
** $p < 0.01$ Significance of difference from control value

TABLE 11.6 GROUP 7 RATS: EFFECT OF VIVONEX AND FLEXICAL FEEDING FOR ONE MONTH ON THE MORPHOLOGY OF RAT JEJUNUM

VH = Villus height (μm) CH = crypt height (μm)

	Control			Vivonex			Flexical		
	VH	CH	CH/VH	VH	CH	CH/VH	VH	CH	CH/VH
	339	107	0.32	534	110	0.21	418	71	0.17
	281	114	0.41	562	91	0.16	474	97	0.20
	417	111	0.27	656	119	0.18	514	92	0.18
	594	130	0.22	424	77	0.18	474	97	0.20
	476	122	0.26	543	105	0.19	571	118	0.21
	409	104	0.25	557	99	0.18	646	119	0.18
Mean ±SEM	419 ±45	115 ± 4	0.29 ±0.03	546 ±30	100 ± 6	0.18 ±0.01 **	516 ±33	99 ± 7	0.19 ±0.01 **

** $p < 0.01$ Significance of difference from control value

TABLE 11.7 GROUP 8 RATS: EFFECT OF VIVONEX AND FLEXICAL FEEDING FOR ONE MONTH ON THE MORPHOLOGY OF RAT JEJUNUM

VH = villus height (μm) CH= crypt height (μm)

	Control (n=12)	Vivonex (n=12)	Flexical (n=12)
Villus Height (μm)	394 ± 23	506* ± 21	495* ± 19
Crypt Height (μm)	117 ± 3	109 ± 5	107 ± 5
Crypt Height / Villus Height	0.31 ±0.02	0.22* ±0.01	0.22* ±0.01

* $p < 0.002$ Significance of difference from Control value

TABLE 11.3

COMBINED RESULTS FOR GROUP 7 AND 8 RATS: EFFECT OF VIVONEX AND FLEXICAL FEEDING FOR ONE MONTH ON THE MORPHOLOGY OF RAT JEJUNUM

Values are Mean ± SEM

	Control			Vivonex			Flexical		
	VH	CH	CH/VH	VH	CH	CH/VH	VH	CH	CH/VH
	377	159	0.42	460	93	0.20	572	144	0.25
	510	149	0.29	418	167	0.40	497	113	0.23
	438	151	0.34	652	109	0.17	438	132	0.30
	228	121	0.53	484	111	0.23	547	130	0.24
	384	144	0.38	393	94	0.24	372	121	0.33
	422	148	0.35	489	169	0.35	539	124	0.23
Mean ±SEM	393 ±38	145 ± 5	0.39 ±0.03	483 ±37	124 ±14	0.27 ±0.04	494 ±31	127 ± 4	0.26* ±0.02

* $p < 0.01$ Significance of difference from control value

TABLE 11.9 GROUP 7 RATS: EFFECT OF ELEMENTAL DIET FEEDING FOR ONE MONTH ON THE MORPHOLOGY OF RAT ILEUM

VH = villus height (μm) CH = crypt height (μm)

	Control			Vivonex			Flexical		
	VH	CH	CH/VH	VH	CH	CH/VH	VH	CH	CH/VH
	402	126	0.31	571	103	0.18	540	103	0.19
	402	115	0.29	563	103	0.18	483	138	0.29
	356	126	0.35	655	103	0.16	483	92	0.19
	322	115	0.36	609	115	0.19	529	103	0.19
	379	161	0.42	460	115	0.25	483	126	0.26
	379	126	0.33	609	126	0.21	575	103	0.18
Mean ±SEM	373 ±12	128 ± 7	0.34 ±0.02	579* ±27	111 ± 4	0.20* ±0.01	516* ±16	111 ± 7	0.22* ±0.02

* $p < 0.01$ Significance of difference from control value

TABLE 11.10 GROUP 8 RATS: EFFECT OF ELEMENTAL DIET FEEDING FOR ONE MONTH ON THE MORPHOLOGY OF RAT ILEUM

VH = villus height (μm) CH = crypt height (μm)

	Control (n=12)	Vivonex (n=12)	Flexical (n=12)
Villus Height (μm)	383 ± 20	531** ± 26	505** ± 17
Crypt Height (μm)	137 ± 5	117* ± 7	119* ± 5
Crypt Height / Villus Height	0.36 ±0.02	0.23** ±0.02	0.24** ±0.01

*$p < 0.05$
**$p < 0.002$ Significance of difference from Control value

TABLE 11.11

COMBINED RESULTS FOR GROUP 7 AND 8 RATS: EFFECT OF VIVONEX AND FLEXICAL FEEDING FOR ONE MONTH ON THE MORPHOLOGY OF RAT ILEUM

Values are Mean ± SEM

Morphological Parameter	Dietary Group	Significance of difference between ileum and jejunum
Villus height	Control Vivonex Flexical	Not significant Not significant Not significant
Crypt height	Control Vivonex Flexical	$p < 0.05$, ileum > jejunum Not significant $p < 0.05$, ileum > jejunum
Crypt height / Villus height	Control Vivonex Flexical	$p < 0.05$, ileum > jejunum Not significant $p < 0.05$, ileum > jejunum

TABLE 11.12

COMPARISON OF THE MORPHOLOGY OF RAT JEJUNUM AND ILEUM IN CONTROL RATS AND THOSE FED VIVONEX AND FLEXICAL

Paired measurements of villus height, crypt height and the crypt height:villus height ratio for the jejunum and ileum of the rats in Groups 7 and 8 were compared using Wilcoxons Signed Ranks test.

CHAPTER 12

DISACCHARIDASE AND ALKALINE PHOSPHATASE ACTIVITY OF RAT JEJUNUM DURING ELEMENTAL DIET FEEDING

INTRODUCTION

Alkaline phosphatase and disaccharidase activities of rat jejunum have been used to measure the function of the jejunum as a result of anatomical and dietary manipulations (Deren et al, 1967; Dolin et al, 1971; Weser et al, 1971; Levine et al, 1974; Dworkin et al, 1976; Ecknauer et al, 1978), and also to examine the effects of different bacterial populations of the gut (Mickelsen, 1962; Dahlquist et al, 1965; Reddy et al, 1968; Mietanen and Hanninen, 1971).

The effects of an elemental diet compared with a normal solid diet on jejunal enzyme activities have not been reported however.

AIM OF STUDY

To investigate the effect of the elemental diets Vivonex and Flexical on the function of rat jejunum by measuring the activity of alkaline phosphatase, lactase, sucrase and maltase in rat jejunum.

METHODS

The effect of elemental diet feeding on jejunal enzyme activities was examined in the rats used for the bile acid metabolism and histological studies described in previous chapters.

The effect of Vivonex feeding for one month was also investigated in five rats fed Vivonex and four fed the control diet (Group 6 rats).

Two groups of twelve rats with equal numbers in each group fed the control diet and Vivonex (Groups 3 and 4) were then studied after three months of feeding. A further group of twelve rats (Group 5), in which half were fed control diet and half fed Flexical, was also studied after three months.

Preparation of Tissue for Enzyme Assays

The rats were sacrificed with an overdose of ether and the abdomen opened. A 10cm segment of jejunum was taken 30-40cm distal to the pylorus (one third of the distance from pylorus to ileo-caecal valve). The segment was rinsed in ice cold sodium chloride solution (154mM), frozen in solid carbon dioxide/methanol and stored at -20°C for up to one week. The 10cm segments were cut into pieces and homogenised in a hand-operated Teflon pestle and tube homogeniser with 5ml of ice-cold water. The homogenate was decanted into a graduated tube and the remaining tissue homogenised and decanted a further three times. The volume was then adjusted and suitable dilutions prepared for assay of protein content (Lowry et al, 1951), disaccharidase activity (Dahlquist, 1964) and alkaline phosphatase activity (Bergmeyer, 1963) as described for rat jejunum by Bolin et al (1971). All assays were done in duplicate.

(a) Protein Content

The alkaline copper reagent was freshly prepared by mixing 50ml of 2% w/v Na_2CO_3 in 0.1N NaOH with 1ml of 0.5% w/v $CuSO_4$ in 1% w/v sodium potassium tartrate solution (Solution A).

Folin and Ciocalteu's phenol reagent (BDH Ltd) was diluted to make it 1N in acid (Solution B).

5ml of solution A was added to 1ml of diluted jejunal homogenate, mixed well and left for 10min. 0.5ml of solution B was then added, mixed rapdily and the optical density read at 727nm after exactly 20min. (Gilford 300N spectrophotometer, Gilford Instrument Laboratories Inc., Oberlin, Ohio). Bovine serum albumin was used as standard. The protein content was expressed as mg protein per cm of jejunum.

(b) Disaccharidase Activity (Lactase, Sucrase and Maltase)

The substrate solutions used were as follows:- Lactose (56mM) in 0.1M maleate buffer pH 5.6;sucrose (56mM) in 0.1M maleate buffer pH 6.0; maltose (56mM) in 0.1M maleate buffer pH 6.0.

Tris-glucose oxidase reagent (TGO reagent) was prepared by dissolving 125mg glucose oxidase (Sigma) in 50ml of Tris buffer (0.5M, pH 7.0) and adding 0.5ml of peroxidase solution (1mg/ml), 0.5ml of o-dianisidine solution (10mg/ml in 95% ethanol, freshly prepared) and 1ml of detergent solution (Triton X-100 diluted 1 in 5 with 95% ethanol). The volume was made up to 100ml with Tris buffer.

0.1ml of diluted jejunal homogenate was incubated at 37°C for 60 min with 0.1ml of each substrate solution and the reaction stopped by immersing in a 65°C water bath for 10 min. Blanks were prepared by mixing homogenate and substrate and immersing in the 65°C water bath immediately. 0.3ml of water was then added to blank and sample tubes and mixed well. Glucose was determined on 0.5ml aliquots of blanks and samples, together with a set of glucose standards (0-50mg glucose), by adding 3ml of TGO reagent and incubating at 37°C for 1 hour. Colour development was measured at 420nm and enzyme activity expressed as Units per mg protein and per cm of jejunum. (1 Unit = 1 μmol substrate hydrolysed per min at 37°C).

(c) Alkaline Phosphatase Activity

The alkaline buffer-substrate solution (0.05M glycine buffer, 5.5mM p-nitrophenylphosphate, pH 10.5) was prepared by dissolving 375mg glycine, 10mg magnesium chloride

($MgCl_2 \cdot 6H_2O$) and 165mg p-nitrophenylphosphate in 42ml 0.1 N NaOH. The pH was then adjusted and the volume made up to 100ml.

The buffer substrate solution (1ml) was preincubated in a water bath at 37°C for 5-10min, and then 0.1ml of diluted jejunal homogenate was added to the sample tubes and the contents mixed. After 30min, 10ml of 0.02N NaOH was added to the sample tubes, to stop the reaction, also to the control tube (1ml of buffer-substrate) for each sample. 0.1ml of homogenate was then added to the control tubes.

The colour development of sample and control tubes, together with standards of p-nitrophenol (0-0.35μmol) was measured at 400nm. The readings for the control tubes (non-enzymic hydrolysis) were subtracted from the sample tube readings and alkaline phosphatase activity expressed as Units per mg protein and per cm of jejunum.
(1 Unit = 1μmol substrate hydrolysed per min at 37°C).

Statistics

Differences between control and elemental diet fed rats were analysed using a two-tailed Mann-Whitney U test (Siegel, 1956).

RESULTS

The disaccharidase activities found for the control rats in this study are in good agreement with the values previously reported for rat jejunum (Bolin et al, 1971; Levine et al, 1974) both for specific activity and activity per cm, and also the relative proportions of the three disaccharidases measured.

The results for the group of rats studied after one month of Vivonex feeding are shown in Table 12.1. Total alkaline phosphatase activity was significantly increased from

1051 ± 37 (Mean ± SEM) in the controls to 1216 ± 37 m Units/cm in the rats fed Vivonex ($p < 0.05$) and specific activity increased from 117.4 ± 14.4 to 179.1 ± 13.8 m Units/mg protein ($p < 0.02$). Lactase specific activity was significantly increased from 17.4 ± 19 in the controls to 32.0 ± 3.2 m Units/mg protein in the rats fed Vivonex ($p < 0.02$), but the increase in total lactase activity per cm of jejunum was not statistically significant. The only other significant change was a decrease in total sucrase activity from 936 ± 67 in the controls to 692 ± 46 m Units/cm in the rats fed Vivonex ($p < 0.02$). Mean maltase activity both per cm and per mg protein was increased in the rats fed Vivonex but not significantly.

After three months of Vivonex feeding a significant increase was found in total alkaline phosphatase activity from 671±27 in the controls (n=12) to 809 ± 50 m Units/cm in the rats fed Vivonex (n=12) ($p < 0.05$), but the increase in specific activity was not significant (Table 12.2). No differences between control and Vivonex fed rats were found after three months of feeding for any of the three disaccharidases expressed either as activity per cm jejunum (Table 12.3) or per mg protein (Table 12.4).

Flexical feeding for three months also increased mean total alkaline phosphatase activity from 651 ± 16 (Mean ± SEM) in the controls (n=6) to 744 ± 50 m Units/cm in the rats fed Flexical (n=6), but the difference was not statistically significant with the small number of rats studied. No change in specific activity was found (Table 12.5). The effect of Flexical feeding on disaccharidase activities is shown in Table 12.6. The only significant difference was a decrease in sucrase specific activity from 39.2 ± 6.6 to 67.4 ± 5.1 m Units/mg protein in the rats fed Flexical ($p < 0.05$).

DISCUSSION.

The generally lower disaccharidase activities found in the control rats for the three month studies, compared with those from the one month study, probably results from the difference in age of the rats. Disaccharidase activity tends to decrease with age (Bolin et al, 1971) and, since the rats were all of comparable age at the start of the studies, it means that those fed for three months were older when the jejunum was sampled for enzyme assays. The older rats may be less susceptible to induction of disaccharidase activity, which could explain the lack of a significant increase in lactase activity for the rats fed Vivonex for three months, in contrast to those fed for one month.

The increased alkaline phosphatase activity per cm of jejunum observed in both groups of Vivonex fed rats suggests an increase in the proportion of functional villous cells in the total cell population of the jejunal mucosa, but the activity of the disaccharidases also located in the brush border cells, does not parallel that of alkaline phosphatase. Alterations of jejunal disaccharidase activity in rats by dietary manipulation have been reported to occur independently of changes in alkaline phosphatase activity (Deren et al, 1967; Bolin et al, 1971; Ecknauer et al, 1973).

As reported in the previous chapter, the crypt height:villus height ratio of the Vivonex-fed rats resembles that of germ-free rats. Disaccharidase activities in germ-free rats were reported to be higher than in conventional rats (Reddy et al, 1968), of similar level in mid-jejunum (Dahlquist et al, 1965) or the same (Nickelsen, 1962). Increased alkaline phosphatase and maltase was reported in specific pathogen free rats compared with conventional rats (Mietanen and Hanninen, 1971). The relationship

between villus height, disaccharidase activity and alkaline phosphatase activity is therefore confused and complex.

The increase in alkaline phosphatase activity in the rats fed Flexical for three months was not statistically significant, which may be a result of the small numbers of rats studied or possibly reflect a true difference between Vivonex and Flexical on jejunal function. Thus although these studies showed some increases in jejunal enzyme activities as a result of Vivonex feeding, particularly an increase in total alkaline phosphatase activity, it was decided to investigate small intestinal function in a more physiological, direct way by perfusing segments of small intestine in vivo.

	Alkaline Phosphatase		Lactase		Sucrase		Maltase	
	Control	Vivonex	Control	Vivonex	Control	Vivonex	Control	Vivonex
Total Activity (mUnits/cm jejunum)	1104	1259	153.3	244.5	807	735	5052	6667
	938	1328	187.3	175.5	1067	514	3370	5927
	1099	1115	160.7	276.3	836	778	3437	3282
	1051	1157	126.0	237.0	1033	730	4370	3437
		1211		161.5		701		4393
Mean ±SEM	1051 * ± 37	1216 ± 37	156.8 ±12.7	219.0 ±21.7	936 ** ±67	692 ±46	4057 ±400	4740 ±673
Specific Activity (mUnits/mg protein)	158.6	209.8	22.0	40.7	116	113	726	1111
	91.7	212.8	18.3	28.1	104	82	329	950
	109.9	142.2	16.1	35.2	84	99	344	419
	109.4	164.4	13.1	33.7	108	104	455	488
		166.3		22.2		96		603
Mean ±SEM	117.4 ** ±14.4	179.1 ±13.8	17.4 ** ±1.9	32.0 ±3.2	103 ± 7	101 ± 7	464 ±92	714 ±135

* $p < 0.05$, ** $p < 0.02$ for control v Vivonex

TABLE 12.1 EFFECT OF VIVONEX FEEDING FOR ONE MONTH ON ALKALINE PHOSPHATASE AND DISACCHARIDASE ACTIVITIES OF RAT JEJUNUM. (GROUP 6 RATS)

	Protein Content (mg/cm jejunum)		Alkaline Phosphatase Activity (mUnits/cm jejunum)		Alkaline Phosphatase (mUnits/mg protein)	
	C	V	C	V	C	V
Group 3	5.68	5.92	437	640	77.0	108.1
	6.40	5.84	731	827	114.2	141.6
	5.36	5.92	587	896	109.5	151.4
	6.88	5.60	720	748	104.7	132.4
	5.68	8.00	661	805	116.4	100.7
	6.16	6.00	779	853	126.4	142.2
Group 4	6.72	7.04	645	656	96.0	93.2
	7.24	7.60	715	720	98.0	94.7
	6.88	7.60	613	1083	89.1	142.5
	6.92	7.08	720	859	103.9	121.1
	7.92	8.40	693	1115	87.5	132.7
	7.04	7.68	747	512	106.1	66.7
Mean ±SEM	6.57 ±0.22	6.89 ±0.28	671 * ±27	809 ±50	102.5 ± 4.0	118.9 ± 7.5

* Control v Vivonex, $p < 0.05$

TABLE 12.2 EFFECT OF VIVONEX FEEDING FOR THREE MONTHS ON PROTEIN CONTENT AND ALKALINE PHOSPHATASE ACTIVITY OF RAT JEJUNUM

C = Control diet V = Vivonex fed

	Lactase (mUnits/cm jejunum)		Sucrase (mUnits/cm jejunum)		Maltase (Units/cm jejunum)	
	C	V	C	V	C	V
Group 3	103.7	74.1	341	405	2.39	2.36
	114.1	93.3	551	477	2.13	2.54
	103.7	74.1	570	407	2.37	2.19
	118.5	85.9	600	403	2.82	2.17
	-	207.4	-	696	-	2.30
	125.9	155.6	582	570	2.56	3.00
Group 4	97.8	104.0	550	596	1.54	2.63
	122.0	120.0	769	353	3.06	1.85
	99.3	151.0	628	682	2.06	2.26
	129.0	126.0	747	534	2.40	2.24
	133.0	132.0	614	769	1.92	2.98
	125.0	51.9	523	468	2.30	1.72
Mean ±SEM	115.6 ± 3.8	114.6 ±12.5	589 ±34	530 ±39	2.32 ±0.13	2.35 ±0.11

All control v Vivonex differences not statistically significant

TABLE 12.3 EFFECT OF VIVONEX FEEDING FOR THREE MONTHS ON DISACCHARIDASE ACTIVITY PER UNIT LENGTH OF RAT JEJUNUM

C = control diet, V = Vivonex fed

	Lactase (mUnits/mg protein)		Sucrase (mUnits/mg protein)		Maltase (mUnits/mg protein)	
	C	V	C	V	C	V
Group 3	18.3	12.5	60.0	68.3	420	398
	17.8	16.0	86.1	81.7	333	435
	18.3	12.5	106.4	68.8	442	370
	17.2	15.3	87.2	72.0	409	388
	-	25.9	-	87.0	-	287
	20.4	25.9	94.5	95.1	415	500
Group 4	14.6	14.7	81.8	84.6	229	374
	16.8	15.8	106.1	46.4	422	244
	14.4	19.8	91.3	89.7	299	297
	18.6	17.8	107.8	75.2	346	316
	16.8	15.7	77.4	91.5	242	355
	17.7	6.8	74.3	61.0	362	225
Mean ±SEM	17.4 ±0.5	16.6 ±1.6	88.5 ±4.5	76.8 ±4.1	357 ±22	349 ±23

All control v Vivonex differences not statistically significant

TABLE 12.4 EFFECT OF VIVONEX FEEDING FOR THREE MONTHS ON DISACCHARIDASE SPECIFIC ACTIVITY OF RAT JEJUNUM

C = control diet V = Vivonex fed

	Protein Content (mg/cm jejunum)		Alkaline Phosphatase (mUnits/cm jejunum)		Alkaline Phosphatase (mUnits/mg protein)	
	C	F	C	F	C	F
	8.08	10.00	629	787	77.6	78.7
	9.76	10.56	637	683	98.9	64.6
	8.48	9.36	667	659	78.6	70.4
	9.68	10.08	608	653	62.8	64.8
	10.48	9.44	645	709	61.6	75.1
	10.56	11.92	720	976	68.2	81.9
Mean ± SEM	9.51 ± 0.42	10.23 ± 0.38	651 ± 16	744 ± 50	72.6 ± 2.9	74.6 ± 5.7

All control v Flexical differences not statistically significant

TABLE 12.5 EFFECT OF FLEXICAL FEEDING FOR THREE MONTHS ON PROTEIN CONTENT AND AIKALINE PHOSPHATASE ACTIVITY OF RAT JEJUNUM (GROUP 5 RATS)

C = control diet F = Flexical fed

	Lactose		Sucrase		Maltase	
	C	F	C	F	C	F
Total activity (mUnits/cm jejunum)	88.9	14.8	711	748	2960	1790
	104.0	59.3	591	726	2550	2260
	78.5	26.7	914	593	2020	1840
	130.0	135.0	975	622	1100	1220
	156.0	72.6	965	553	410	650
	104.0	23.3	904	919	2000	1220
Mean ±SEM	110.2 ±11.6	89.9 ±33.3	843 ±64	694 ±55	1840 ±380	1500 ±240
Specific activity (mUnits/mg protein)	11.0	1.5	88.0	74.8	367	179
	10.6	5.6	60.6	68.7	261	214
	9.3	2.9	107.8	63.3	237	196
	13.5	13.2	100.7	61.7	114	121
	14.9	7.7	92.6	58.5	39	69
	9.8	19.6	85.6	79.5	189	103
Mean ±SEM	11.5 ±0.9	8.4 ±2.8	89.2 ±6.6	*67.4 ±3.1	201 ±47	147 ±24

* $p < 0.05$, Control v Flexical. All other differences not statistically significant

TABLE 12.6 EFFECT OF FLEXICAL FEEDING FOR THREE MONTHS ON DISACCHARIDASE ACTIVITY OF RAT JEJUNUM (GROUP 5 RATS)

C = Control diet F = Flexical fed

CHAPTER 13

STUDY BY AN IN VIVO PERFUSION TECHNIQUE OF THE EFFECTS OF DIETARY DIET FEEDING ON THE FUNCTION OF RAT JEJUNUM

INTRODUCTION

It has been demonstrated (Chapter 11) that elemental diet feeding in normal rats results in a decreased crypt height to villus height ratio, mainly as a result of increased villus height. Measurement of jejunal brush border enzyme activities (Chapter 12) showed an increase in total alkaline phosphatase activity suggestive of an increased absorptive area or function, but no increase in disaccharidase activity was found. The disaccharidases may be specifically affected by the nature of the carbohydrate content of the diet (Bolin et al, 1971) and an alternative method of measuring jejunal function during elemental diet feeding was sought.

Water absorption represents a basic function of the small bowel and measurement of this in vivo provides a good assessment of small bowel function. Absorption of specific dietary components may be influenced by the nature of the diet and not represent the overall function of the bowel.

Various methods have been reported for measurement of in vivo absorption in the rat. The method reported by Sheff and Smyth (1955) introduced the use of a recirculation - perfusion technique for cannulated loops of intestine in anaesthetised rats, employing a gas-lift to circulate the fluid. This was modified by Dowling and Booth (1967), who studied absorption in rats that had been cannulated and allowed to recover from anaesthesia in a restraining cage. Other workers have studied absorption in anaesthetised rats but employed a pump to recirculate the perfusate, rather than a gas-lift, and so controlled the flow rate more precisely (Lewis and Fordtran, 1975; Schneuer et al, 1973). However, the original gas-lift recirculation in anaesthetised rats is used by many workers (Loese, 1974; Debnam and Levin, 1975;

Davidson and Leese, 1977). In allthese methods it is disappearance from the gut lumen which is measured. A more sophisticated technique of vascular perfusion, which allows both disappearance from the gut lumen and appearance in the blood vessels to be quantitated, has recently been developed and successful preparations achieved by some workers (Jacobs et al, 1966; Counar and McJoll, 1975; Bronk and Ingham, 1976).

Water absorption can be determined by the use of a non-absorbable marker. Phenol red (Fisher and Gardner, 1974; Ecknauer et al, 1978) and polyethylene glycol with a molecular weight of 4000 (Gleeson et al, 1972; Lewis and Fordtran, 1975) have been used in the rat. The use of ^{14}C labelled polyethylene glycol 4000 improves the accuracy of measurement in perfusion fluids (Batt and Peters, 1976; Hughes et al, 1973). Absorption of water increases the concentration of the marker in the perfusion fluid. Absorption of solutes can be calculated by measuring changes in concentration and correcting for the change in volume that has occurred.

AIM OF STUDY

To study the effect of elemental diet feeding on the function of rat jejunum by measuring water and glucose absorption utilising an in vivo, gas-lift recirculation perfusion technique in anaesthetised rats and ^{14}C labelled polyethylene glycol 4000 as a non-absorbable marker.

METHODS

Groups Studied

Two groups of eighteen rats were studied at different times. Equal numbers in each group were fed the control diet, Vivonex and Flexical as before (Charter 6). Since faecal collections

were not required, conventional rat cages were used rather than metabolic cages. Thus, the number of rats was not restricted to twelve and all three diets were studied at the one time. The cages had wide mesh bottoms to limit coprophagy and the animal room was illuminated from 6 am to 6 pm.

The diets were fed for four weeks and then the rats were perfused over a period of one to two weeks, two or three perfusions being performed each day.

The first group of rats (Group 7) was perfused during the day with equal numbers from each dietary treatment being perfused at 10 am, 1 pm and 3 pm.

The second group of rats (Group 8) was perfused at night with equal numbers from each dietary treatment being perfused at 10 pm and 12 midnight. None of the rats were fasted before perfusion.

Composition of Perfusion Solution

The composition of the perfusion solution is shown in Table 13.1, and consists of modified Krebs-Ringer bicarbonate (Bronk and Leese, 1974) with 11.1mM glucose added (Leese et al, 1976) and ^{14}C labelled polyethylene glycol 4000 (PEG 4000) as non-absorbable marker.

Perfusion Technique

The apparatus used (Sheff and Smyth, 1955) is shown in Figure 13.1. The perfusion fluid (50ml) was equilibrated with 5% CO_2 in O_2 by circulating the fluid through the apparatus, with a glass inset in place of the jejunal loop, during the time that each rat was being anaesthetised and prepared.

Rats were weighed and anaesthetised with sodium pentobarbitone (Sagatal; May and Baker Ltd, Dagenham) by injecting 60mg/Kg intraperitoneally. A rectal thermometer was inserted and the abdomen opened by a ventral mid-line incision, avoiding major blood vessels. A proximal glass canula (4mmOD) was inserted just beyond the ligament of Treitz, where the loop of jejunum re-emerges, and a second transection of jejunum made 20-30cm distally. The jejunal segment was then rinsed through with 10ml of sodium chloride (154mM, saline) to remove food debris. A second canula was then tied into the distal end and the segment dried by blowing through 20ml of air from a syringe. Great care was taken not to damage the mesenteric blood vessels and the transections were made avoiding the larger vessels.

The cannulated jejunal loop was then connected to the perfusion circuit, taking care not to lose any volume, by switching off the gas and elevating the ends of the tubing during connection. The cannulated loop was replaced inside the abdomen and a pad moistened with saline placed over the closed abdomen to prevent the tissue drying out. With practise it was possible to cannulate the jejunum and start the perfusion within five minutes of opening the abdomen. Fluid was circulated through the loop for 60 min. (flow rate averaged 25ml/min) and body temperature was maintained at 36.5-37°C with the aid of a lamp and the rectal thermometer. Samples of fluid (0.2ml) were taken at 10 min intervals from the reservoir for measurement of PEG and glucose concentrations and a final 0.5ml samples was taken at 60 min.

The rat was then sacrificed by incision of the heart and the perfused segment (with fluid still circulating) freed from the mesentery and the length measured. The length of the remaining proximal and distal small intestine (to the ileo-caecal

valve) was then measured and 5cm segments taken for histological examination, as described in Chapter 11.

Measurement of PEG and Glucose

Four aliquots (0.1ml) of initial fluid, and final perfusate in the resevoir at 60 min, were taken for counting in addition to one (0.1ml) aliquot at 10, 20, 30, 40 and 50 min. Duplicate aliquots (20µl) were analysed for glucose from each ten minute sample, in addition to the initial and final samples.

10ml of 'Instagel' scintillant (Packard Instrument Ltd.) was added to the 0.1ml samples and ^{14}C PEG determined using a Tricarb liquid scintillation counter (Packard). Quenching was negligible but was corrected for by automatic external standardisation. Counting efficiency was 30-35%.

Glucose was determined on the 20µl aliquots by incubating for 60 min with 3 ml of Tris-glucose oxidase reagent, as described for estimation of disaccharidases in Chapter 12.

Calculations

The term 'absorption' is used to describe disappearance from the gut lumen (i.e. net absorption and utilization). The total volume of water absorbed at each 10 minute interval was calculated as follows and expressed as µl water absorbed per cm of jejunum.

Volume at time t, $V_t = V_i \times \frac{dpmi}{dpmt}$

Vol absorbed at time t = $V_i - V_t = V_i \left(1 - \frac{dpmi}{dpmt}\right)$

Vol absorbed per cm of jejunum at time t = $\frac{V_i - V_t}{L}$

Where V_i = volume initially (i.e. 50ml)

V_t = volume at time t

dpmi = ^{14}C PEG dpm in 0.1ml initially

dpmt = ^{14}C PEG dpm in 0.1 ml at time t

L = length of perfused segment (cm)

Similarly, the total amount of glucose absorbed at each 10 min interval was calculated and expressed as μmol glucose absorbed per cm of jejunum.

$$\mu\text{mol glucose absorbed per cm of jejunum at time t} = \frac{(V_i \times C_i) - (V_t \times C_t)}{L}$$

Where C_i = glucose concentration initially (μmol/ml)

C_t = glucose concentration at time t

L = length of perfused segment

Sample size at the 10 min intervals was kept to a minimum so that the slight decrease in total ^{14}C PEG dpm remaining after each sample is taken has a negligible effect on the calculation and is ignored in the calculations.

i.e. True [PEG] after 10 min sample

$$= \frac{dpm_i - dpm_s}{50 - (\text{vol absorbed} + 0.2 \text{ ml})}$$

where dpm_i = total dpm initially

dpm_s = dpm removed in 10 min sample

and not:- $\frac{dpm_i}{50 - \text{vol absorbed}}$

Statistics

The significance of differences between the three dietary treatments were analysed using Wilcoxon's sum of ranks test (Langley, 1963). In some instances differences which were just not significant as judged by the ranking test, did show significance when analysed by a t-test, but since the latter makes assumptions about the distribution of data which are not necessarily true, the ranking test was adhered to throughout.

RESULTS

Numerous perfusions were performed before this study was done to gain competence with the technique and achieve values for absorption comparable to those reported for control rats in the literature. It was confirmed histologically that the jejunum was not damaged during perfusion (Chapter 11) even with the relatively high flow rate necessitated by the gas-lift recirculation technique.

The initial and final body weights of the rats in both groups 7 and 8 are shown in Table 13.2. The only significant difference found was for final body weight between control (n=12) and Vivonex fed (n=12) rats when the two groups were combined ($p < 0.05$). Table 13.3 shows the length of small intestine from pylorus to ileo-caecal value for the two groups of rats. Again the difference between Vivonex fed and control rats was just significant ($p < 0.05$) when the two groups were combined, with a slightly shorter small intestinal length in the rats fed Vivonex. There was no significant difference between the three dietary treatments in the length of jejunum perfused (measured after perfusion) nor in the distance of the perfused segment from the pylorus (Table 13.4).

Table 13.5 shows the water absorption by group 7 rats for the three dietary treatments at the ten minute intervals. No difference was found between control and Vivonex fed rats but the rats fed Flexical absorbed less water than the controls, the difference being significant from 40 min onwards ($p < 0.05$ in each case). Glucose absorption (Table 13.6) showed a similar pattern with significantly less absorption by the rats fed Flexical compared with the controls at 50 min ($p < 0.01$) and 60 min ($p < 0.05$). No results are available for the fourth control and fifth Vivonex fed rat in group 7, both of which died under the anaesthetic during

perfusion. Greater losses were encountered for Group 8 rats which were perfused at night, and in one instance this was due to the fact that the nocturnal rats were more lively at night and harder to inject. No attempt was made to perfuse the sixth rat fed Flexical, after five had been successfully perfused.

The results for water and glucose absorption in group 8 rats are shown in Tables 13.7 and 13.8. Again mean water and glucose absorption was less in the rats fed Flexical, but the small numbers preclude statistical analysis. Mean water and glucose absorption by the control rats was less at night (Group 8) than during the day (Group 7), but the difference was not statistically significant.

The results for the two groups have been combined and the mean values (together with SEM) are shown graphically for water absorption in Figure 13.2 and glucose absorption in Figure 13.3. The rats fed Flexical (n=11) absorbed significantly less water than the controls (n=9) at 40 and 60 min ($p < 0.01$ in each case). The only significant difference in glucose absorption observed was between the rats fed Vivonex and Flexical at 40 min ($p < 0.05$), the Flexical fed rats absorbing less. It can be seen (Fig.13.2) that a greater lag phase in water transport was observed for the rats fed Flexical than for the other two diets. Water and glucose absorption between 30 and 60 min, when a steady state had been achieved, was therefore calculated and the results are shown in Tables13.9 and 13.10. The Flexical fed rats absorbed less water than the controls ($p < 0.01$) and the Vivonex fed rats ($p < 0.05$) between 30 min and 60 min. Thus the rate of absorption, as well as total absorption, was less for the rats fed Flexical. For glucose absorption, the difference between control and Flexical fed rats was significant ($p < 0.05$) for the 30-60 min period of perfusion.

..o significant differences between the control and Vivonex fed rats were found.

DISCUSSION

The results for water absorption in the control rats (165-219μl $cm^{-1}hr^{-1}$) are comparable to those reported in the literature for in vitro perfusions. Fisher and Gardner (1974) reported a mean water absorption for jejunal segments in vitro of 179 μl $cm^{-1}hr^{-1}$ at the glucose concentration (11.1mM) used in this study, and 132 μl $cm^{-1}hr^{-1}$ was reported for jejunum plus ileum in vitro (Gardner et al, 1978). Water absorption during the day and night in vitro ranged from 130 to 219 μl $cm^{-1}hr^{-1}$ for the entire small intestine (Fisher and Gardner, 1976).

A glucose concentration of 11.1mM was used in this study since water absorption in vitro was maximal at this concentration (Fisher and Gardner, 1974) and it is equivalent to the normal plasma concentration (Leese 1974).

It has been demonstrated (Fisher and Gardner, 1976), that water absorption by the entire rat small intestine in vitro is 50-60% higher at night than during the day. Group 3 rats were therefore perfused at night to establish whether a difference between control and Vivonex fed rats would become apparent. However, no night-time increase in water absorption for the jejunum of the control rats, when perfused in vivo, was demonstrated and, as in group 7, no significant difference between the control and Vivonex fed rats was found. The absence of a night-time increase in water absorption found in this study may be due to the different perfusion technique employed and also to the fact that jejunal segments, rather than the entire small intestine, were studied. No difference in water absorption could be demonstrated between control rats and those fed Vivonex, but the rats fed Flexical showed a consistently

lower water absorption, which was significantly less than for the controls at 40 and 60 min but not significantly different from the Vivonex fed rats. The results for glucose absorption paralleled those for water absorption but may reflect utilization of glucose by the jejunum rather than true absorption.

The results of this study have been expressed in terms of unit length of jejunum as a measure of overall function. The total length of the small intestine for the rats fed the elemental diets tended to be less than for the controls, although this was only significant for the Vivonex fed rats when groups 7 and 8 were combined, which means that the slightly lower water absorption per cm of jejunum in the elemental diet fed rats is not compensated for by a greater length of bowel. It is not technically possible to perfuse segments much longer than 30 cm in vivo, so that the overall function of the entire small bowel cannot be assessed by a single perfusion. It is possible, that perfusion of ileal segments would reveal differences between the control and Vivonex fed rats. The ratio of crypt height to villus height was also decreased in the ileum of rats fed the elemental diets (Chapter 11).

The results of this study indicate that the morphological changes produced by one month of elemental diet feeding are not accompanied by an increase in jejunal function, as measured by water and glucose absorption in vivo. Similar results have been reported for the effect of Vivonex perfusion after Thiry-Vella bypass of jejunum in the dog. (Jacobs et al, 1975). Villus height was significantly increased but no significant increase in galactose absorption was observed, although the mean value was increased. Similar dissociations of villus height and absorptive capacity have been reported in other experimental situations.

Dilution of the diet with unabsorbed bulk (kaolin) produced enhanced in vivo absorption of glucose but no change in villus height in the rat (Dowling et al, 1967), and semi-starvation resulted in an increased absorption of glucose and histidine even though the small bowel showed marked atrophy (Kershaw et al, 1960). Sarcoma bearing rats also showed increased absorption of glucose and histidine along with wasting of the small intestine and a 20% decrease in food consumption (Wiseman et al, 1959). In all these situations nutritional demand is increased as a result of dietary stress.

In other situations increased villus height is accompanied by increased absorptive function. In the pregnant rat increased food intake is associated with an increase in both villus height and glucose absorption to cope with the increased nutritional demand (Larralde et al, 1966) and, after partial resection, the remaining small bowel shows increased villus height and absorptive capacity per unit length (Dowling and Booth, 1967). Since the nutritional requirements of the animal are being served by a reduced length of bowel, the net demand per length of intestine is also increased in this situation.

Thus, nutritional demand may be a more important determinant of absorptive function per unit length of intestine than surface area, as indicated by villus height, which would explain the lack of increased absorptive function in the jejunum of the rats fed the elemental diets in this study.

Although oral food intake and luminal nutrition are very important (Gleeson et al, 1972; Levine et al, 1974; Jacobs et al, 1975; Feldman et al, 1976), and pancreatic and biliary secretions may have direct effects on small bowel structure and function (Altmann, 1971; Weser et al, 1977), humoral mediators have also

been implicated. Indirect effects of luminal nutrition have recently been demonstrated on by-passed intestine (Dworkin et al, 1976; Adams et al, 1973), and in the dog intravenous cholecystokinin and secretin prevent loss of small bowel structure and function during intravenous nutrition (Hughes et al, 1978). Chronic glucagon administration enhances active transport by intestinal rings of the rat (Rudo and Rosenberg, 1973). Differences in the composition of the two elemental diets used in this study may alter the hormonal status of the rat in different ways and account for the reduced absorption observed in the rats fed Flexical.

In the normal intact rat the increase in villus height and reduced crypt height to villus height ratio, which results from one month of elemental diet feeding, is not accompanied by an increase in absorptive function of the jejunum. This may be due to the fact that nutritional demand is not increased, and the differences in effect between the two elemental diets may be due to their different composition.

FIGURE 13.1 PERFUSION OF RAT JEJUNUM IN VIVO

The perfusion fluid is circulated by a gas-lift with 5% CO_2 in O_2. The gas enters the fluid stream via a syringe needle in the side arm below the vertical tube.
Fluid in the reservoir (right) and vertical tube (left) is maintained at 37°C with a water jacket.
Samples (0.2 ml) were taken at 10 min. intervals from the reservoir for counting and glucose assay.

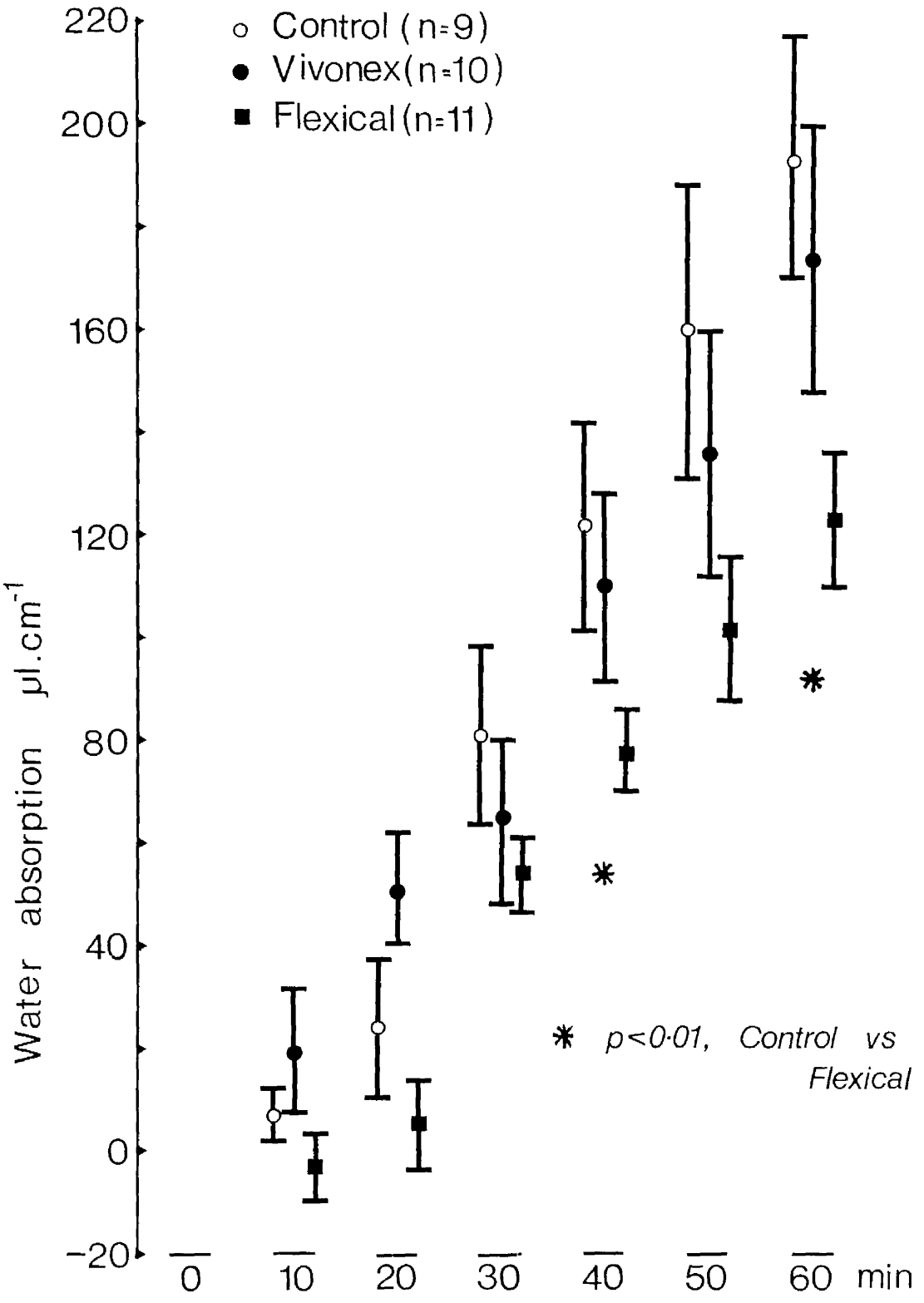


FIGURE 13.2. JEJUNAL WATER ABSORPTION MEASURED AT 10 min INTERVALS DURING PERFUSION. MEAN ± SEM FOR THE RATS OF GROUPS 7 AND 8 COMBINED

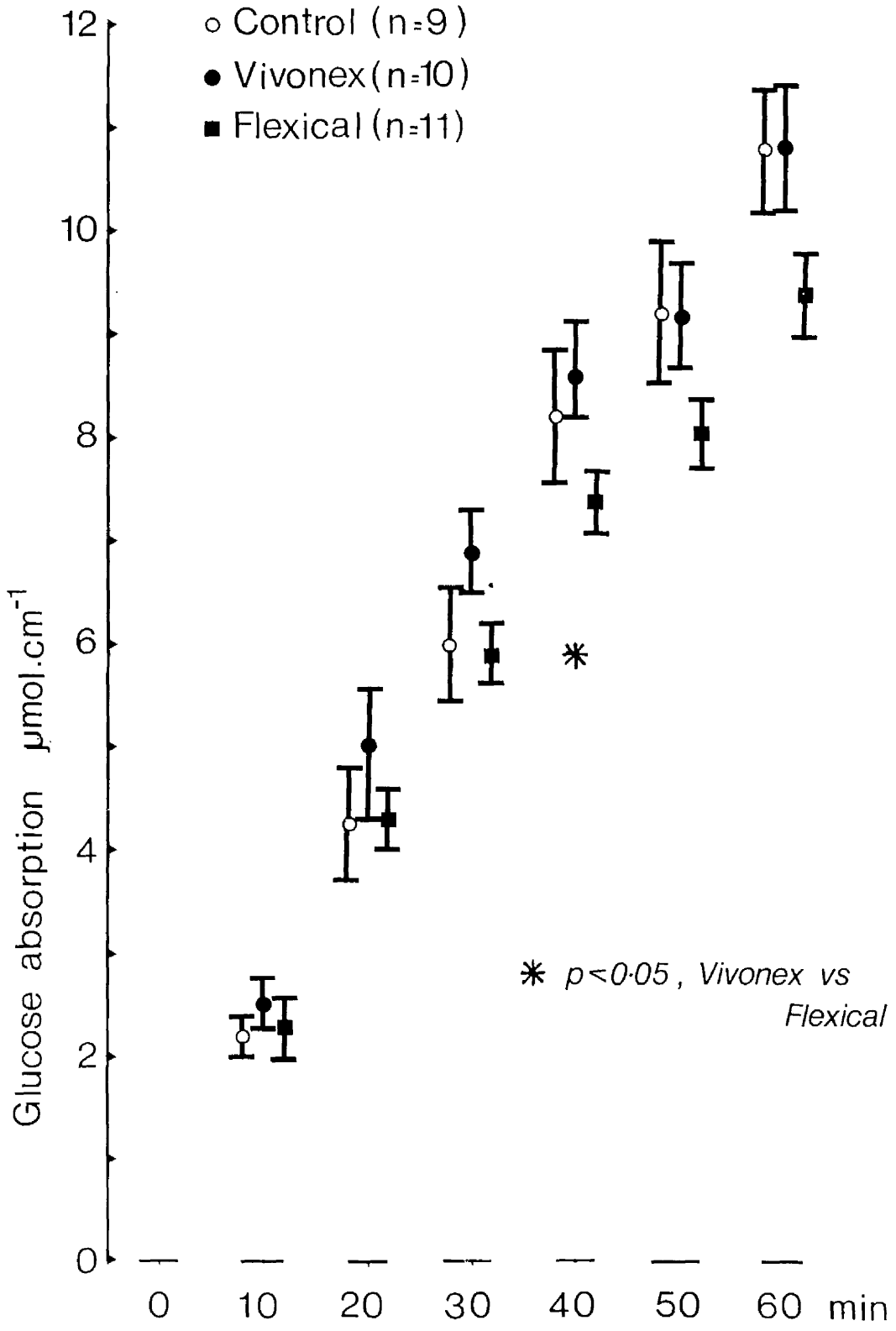


FIGURE 13.3. JEJUNAL GLUCOSE ABSORPTION MEASURED AT 10 min INTERVALS DURING PERFUSION. MEAN ± SEM FOR THE RATS OF GROUPS 7 AND 8 COMBINED

Component	mmol/l
NaCl	118
$NaHCO_3$	25
KCl	4.74
$MgSO_4 \cdot 7H_2O$	1.19
KH_2PO_4	1.17
$CaCl_2 \cdot 6H_2O$	1.70
Polyethylene glycol 4000 (+10μCi/l of ^{14}C labelled polyethylene glycol 4000)	1.25
Glucose	11.1

Solution equilibrated with 95% Oxygen, 5% carbon dioxide (v/v)

TABLE 13.1

COMPOSITION OF PERFUSATE USED FOR IN VIVO PERFUSION OF RAT JEJUNUM

	Rat Body Weight (g)					
	Control		Vivonex		Flexical	
Group 7	Initial	Final	Initial	Final	Initial	Final
	155	335	160	415	140	355
	145	350	145	395	155	335
	150	425	140	420	145	405
	140	410	145	420	140	425
	160	415	155	455	160	400
	160	405	150	412	155	455
Mean ± SEM	152 ± 3	390 ±15	149 ± 3	420 ± 8	149 ± 4	396 ±18
Group 8	135	345	195	375	180	343
	175	325	180	400	140	365
	145	350	100	342	195	375
	185	360	130	405	210	445
	135	345	180	435	130	375
	205	375	195	467	125	375
Mean ±SEM	172 ±11	350 ± 7	172 ±15	404 ±13	172 ±13	332 ±17
Group Mean 7+8 ±SEM	162 ± 6	370 ±10	161 ± 8	412* ±10	161 ± 7	389 ±12

* $p < 0.05$ for Vivonex v Control final body weight (Groups 7 and 8 combined).

TABLE 13.2

INITIAL AND FINAL BODY WEIGHTS (g) FOR GROUP 7 AND 8 RATS (4-6 WEEKS OF FEEDING)

	Length of Small Intestine (cm)		
	Control	Vivonex	Flexical
Group 7	127.0 113.0 132.5 143.0 130.5 136.5	125.5 119.5 122.5 125.5 133.0 120.5	126.5 120.5 132.5 123.5 122.5 136.5
Mean ± SEM	130.4 ± 4.1	124.4 ± 2.0	127.0 ± 2.6
Group 8	136.0 125.0 141.5 - 139.5 139.0	130.0 122.0 132.0 125.0 136.5 132.0	133.0 129.0 122.5 131.0 123.5 130.5
Mean ± SEM	136.2 ± 2.9	129.6 ± 2.2	129.0 ± 1.5
Groups 7+8 Mean ± SEM	133.1 ± 2.7	127.0* ± 1.6	128.0 ± 1.4

* $p < 0.05$ for Vivonex v Control (Groups 7 and 8 combined)

TABLE 13.3

EFFECT OF ELEMENTAL DIET FEEDING ON THE LENGTH OF SMALL INTESTINE OF GROUP 7 AND 8 RATS

Distance in cm from pylorus to ileo-caecal valve

	Length of Perfused Segment and Distance from Pylorus(cm)					
	Control		Vivonex		Flexical	
	PS	D	PS	D	PS	D
Group 7	30.0	11	32.5	19	32.5	15
	24.0	15	29.5	28	34.5	21
	36.5	24	28.5	16	20.5	16
	-	-	17.5	14	23.5	21
	30.5	28	-	-	23.5	14
	27.5	24	24.5	16	29.5	17
Mean ± SEM	29.7 ±2.1	20 ±3	26.5 ±2.6	19 ±3	27.3 ±2.3	17 ±1
Group 8	22.0	18	24.0	17	31.0	13
	-	-	21.0	17	17.0	21
	17.5	20	-	-	23.5	19
	-	-	22.0	17	22.0	19
	31.5	19	30.5	18	27.5	15
	28.0	16	19.0	21	-	-
Mean ± SEM	24.8 ±3.1	18 ±1	23.3 ±2.0	18 ±1	24.2 ±2.4	17 ±2
Group 7 + 8 Mean ± SEM	27.5 ±1.9	19 ±2	24.9 ±1.6	18 ±1	25.9 ±1.7	17 ±1

PS = Length of perfused segment (cm)
D = Distance from pylorus to segment (cm)

No significant differences between the three dietary treatments for group 7 or 8 or the two groups combined.

TABLE 13.4

LENGTH OF PERFUSED SEGMENT OF JEJUNUM (CM) AND DISTANCE FROM PYLORUS TO SEGMENT (CM) FOR GROUP 7 AND GROUP 8 RATS

Time	Water Absorption ($\mu l.cm^{-1}$)		
	Control	Vivonex	Flexical
10 min	15.7	-5.0	21.2
	9.3	32.7	13.1
	4.3	38.7	15.0
	-	8.6	-2.2
	-2.6	-	-18.2
	-11.4	-40.9	-9.0
Mean ±SEM	3.1 ±4.7	6.8 ±14.3	3.3 ±6.3
20 min	-27.6	11.1	35.3
	44.5	75.2	22.4
	67.3	90.1	19.0
	-	82.0	-70.3
	37.6	-	-10.3
	60.6	8.0	-16.9
Mean ±SEM	36.5 ±16.9	53.3 ±18.0	-3.5 ±15.7
30 min	41.2	65.7	76.9
	92.5	130.4	89.5
	184.2	157.9	54.5
	-	49.9	47.6
	54.5	-	25.1
	97.4	18.2	49.3
Mean ±SEM	94.0 ±25.0	84.4 ±25.9	57.2 ±9.4

TABLE 13.5

EFFECT OF ELEMENTAL DIET FEEDING FOR ONE MONTH ON WATER ABSORPTION BY RAT JEJUNUM IN VIVO. GROUP 7 RATS - DAYTIME PERFUSION

Time	Water Absorption ($\mu l.cm^{-1}$) Control	Vivonex	Flexical
40 min	84.1	145.4	76.2
	173.7	189.5	134.6
	239.5	203.5	59.4
	-	98.1	68.9
	109.8	-	65.6
	142.3	59.7	54.5
Mean ±SEM	149.9 ±27.0	139.2 ±27.1	76.5* ±12.0
50 min	120.8	178.0	90.5
	239.7	227.0	188.6
	293.0	268.0	120.5
	-	134.9	119.2
	157.8	-	72.0
	216.2	71.4	84.4
Mean ±SEM	205.5 ±30.3	175.9 ±34.4	112.5* ±17.1
60 min	148.7	179.1	129.1
	284.1	272.3	226.7
	325.7	333.5	124.6
	-	193.1	119.3
	184.6	-	97.1
	136.6	126.9	106.9
Mean ±SEM	215.9 ±37.7	221.0 ±36.5	134.0* ±19.2

* $p < 0.05$ for Control v Flexical

TABLE 13.5/CONTINUED

Time	Glucose Absorption (mmol cm^{-1})		
	Control	Vivonex	Flexical
10 min	2.27	1.45	1.47
	1.65	2.34	1.03
	1.60	3.70	1.07
	-	1.99	3.62
	2.87	-	1.08
	2.52	2.84	2.10
Mean ±SEM	2.18 ±0.25	2.46 ±0.39	1.73 ±0.41
20 min	3.60	1.96	3.92
	4.20	4.49	3.21
	5.94	5.65	2.91
	-	8.32	6.08
	5.96	-	4.40
	6.19	4.86	4.23
Mean ±SEM	5.18 ±0.53	5.06 ±1.02	+4.12 -0.46
30 min	5.45	4.41	5.69
	6.20	6.98	4.99
	6.70	8.22	4.00
	-	8.19	7.35
	6.70	-	5.85
	8.22	7.21	6.73
Mean ±SEM	6.77 ±0.46	7.00 ±0.69	5.77 ±0.49

TABLE 13.6

EFFECT OF ELEMENTAL DIET FEEDING FOR ONE MONTH ON GLUCOSE ABSORPTION BY RAT JEJUNUM IN VIVO.

GROUPS 7 RATS - DAYTIME PERFUSION

Time	Glucose Absorption (μmol.cm^{-1}) Control	Vivonex	Flexical
40 min	7.31	6.63	6.60
	8.28	8.38	6.97
	11.15	10.10	6.38
	-	10.69	7.62
	9.32	-	8.63
	9.85	8.49	8.05
Mean ±SEM	9.18 ±0.66	8.86 ±0.72	7.38 ±0.36
50 min	9.34	8.06	7.67
	9.14	8.75	7.56
	12.28	11.53	7.67
	-	11.52	8.59
	10.23	-	9.13
	10.68	8.92	8.70
Mean ±SEM	10.33 ±0.56	9.75 ±0.74	8.22** ±0.27
60 min	9.79	8.95	8.67
	11.24	10.80	8.89
	13.59	12.96	8.40
	-	14.08	9.90
	12.25	-	10.85
	11.51	12.25	10.01
Mean ±SEM	11.67 ±0.62	11.81 ±0.89	9.45* ±0.39

* $p < 0.05$, ** $p < 0.01$ for Control v Flexical

TABLE 13.6/CONTINUED

Time	Water Absorption ($\mu l.cm^{-1}$) Control	Vivonex	Flexical
10 min	-	67.0	4.5
	-	91.7	16.6
	34.9	-	-62.2
	-	-13.7	- 6.0
	4.0	1.5	-15.8
	-3.5	13.3	-
Mean ±SEM	11.8 ±11.8	32.0 ±20.2	-12.6 ±13.5
20 min	-38.0	47.0	7.2
	-	95.6	5.9
	23.7	-	-7.1
	-	2.0	15.2
	21.2	40.4	40.2
	12.2	58.0	-
Mean ±SEM	4.8 ±14.5	48.6 ±15.1	12.3 ±7.8
30 min	11.0	3.0	30.5
	-	108.4	94.3
	106.0	-	36.2
	-	12.0	61.1
	60.0	51.0	27.5
	-	44.3	-
Mean ±SEM	59.0 ±27.4	43.7 ±18.6	49.9 ±12.6

TABLE 13.7

EFFECT OF ELEMENTAL DIET FEEDING FOR ONE MONTH ON WATER ABSORPTION BY RAT JEJUNUM IN VIVO. GROUP 8 RATS - NIGHTIME PERFUSION

Time	Water Absorption ($\mu l.cm^{-1}$) Control	Vivonex	Flexical
40 min	80.0	29.0	48.6
	-	149.9	47.3
	68.8	-	36.5
	-	50.3	80.5
	71.4	61.3	76.1
	-	109.9	-
Mean ±SEM	73.4 ±3.4	80.1 ±21.9	57.8 ±8.7
50 min	99.0	-14.8	98.3
	-	146.7	-
	46.1	-	21.1
	-	70.2	136.0
	111.3	131.6	93.5
	-	133.9	-
Mean ±SEM	85.5 ±20.0	93.5 ±30.2	87.2 ±24.0
60 min	122.2	51.9	92.6
	-	167.0	118.2
	173.4	-	72.5
	-	102.6	178.6
	139.0	135.3	89.0
	226.5	177.1	-
Mean ±SEM	165.3 ±23.0	126.8 ±22.8	110.2 ±18.6

TABLE 13.7/CONTINUED

Time	Glucose Absorption (μmol.cm^{-1})		
	Control	Vivonex	Flexical
10 min	-	2.92	1.95
	-	3.11	3.05
	2.59	-	2.46
	-	1.91	3.96
	1.85	1.44	3.01
	1.99	3.27	-
Mean ±SEM	2.14 ±0.23	2.53 ±0.36	2.89 ±0.34
20 min	1.06	4.08	3.47
	-	5.77	4.97
	4.34	-	3.70
	-	4.54	5.62
	3.68	3.12	4.78
	3.58	6.70	-
Mean ±SEM	3.16 ±0.72	4.84 ±0.63	4.51 ±0.41
30 min	2.98	7.13	5.36
	-	7.09	7.07
	5.90	-	5.60
	-	6.89	7.07
	5.06	5.28	5.10
	-	7.81	-
Mean ±SEM	4.65 ±0.87	6.84 ±0.42	6.04 ±0.43

TABLE 13.8

EFFECT OF ELEMENTAL DIET FEEDING FOR ONE MONTH ON GLUCOSE ABSORPTION BY RAT JEJEUNUM IN VIVO.

GROUP 8 RATS - NIGHTIME PERFUSION

Time	Glucose Absorption (μmol.cm^{-1}) Control	Vivonex	Flexical
40 min	5.40	8.69	6.15
	-	8.42	8.46
	7.58	-	6.63
	-	8.99	8.68
	6.96	6.76	6.77
	-	9.60	-
Mean ±SEM	6.65 ±0.65	8.49 ±0.48	7.34 ±0.52
50 min	6.13	10.39	6.97
	-	8.11	-
	7.61	-	6.35
	-	7.50	10.36
	8.29	7.04	7.87
	-	10.02	-
Mean ±SEM	7.34 ±0.64	8.61 ±0.67	7.89 ±0.88
60 min	7.95	11.15	7.87
	-	9.66	10.02
	9.50	-	7.65
	-	10.18	11.79
	9.42	7.61	9.00
	12.24	11.70	-
Mean ±SEM	9.78 ±0.89	10.06 ±0.71	9.27 ±0.76

TABLE 13.8/CONTINUED

	Water Absorption 30 to 60 min ($\mu l.cm^{-1}$)		
	Control	Vivonex	Flexical
Group 7	107.5	113.4	52.2
	191.6	141.9	137.2
	141.5	175.6	70.1
	-	143.2	71.7
	130.1	-	72.0
	118.8	108.7	57.6
Group 8	133.2	48.6	62.1
	-	58.6	23.9
	67.5	-	36.3
	-	90.6	117.5
	79.0	84.3	61.5
	160.0	132.8	-
Mean ±SEM	125.5 ±12.8	109.8* ±12.7	69.3** ±9.8

** $p < 0.01$ for Control v Flexical
* $p < 0.05$ for Vivonex v Flexical

TABLE 13.9

EFFECT OF ELEMENTAL DIET FEEDING ON WATER ABSORPTION

BY RAT JEJUNUM DURING THE SECOND HALF-HOUR OF PERFUSION

	Glucose Absorption 30-60 min (μmol.cm^{-1})		
	Control	Vivonex	Flexical
Group 7	4.34	4.54	2.99
	5.04	3.82	3.90
	6.59	4.75	4.40
	-	5.89	2.55
	5.25	-	5.00
	3.29	5.04	3.28
Group 8	4.97	4.02	2.52
	-	2.57	2.96
	3.60	-	2.05
	-	3.29	4.73
	4.36	2.33	3,90
	-	3.90	-
Mean ±SEM	4.68 ±0.37	4.02 ±0.35	3.48* ±0.29

* $p < 0.05$ for Control v Flexical

TABLE 13.10

EFFECT OF ELEMENTAL DIET FEEDING ON GLUCOSE ABSORPTION BY RAT JEJUNUM DURING THE SECOND HALF-HOUR OF PERFUSION

CHAPTER 14

VIVONEX SUPPLEMENTATION IN UNTREATED ADULT COELIAC DISEASE: EFFECTS ON JEJUNAL STRUCTURE AND FUNCTION

INTRODUCTION

"Coeliac disease is a condition characterised by a lesion of the small intestinal mucosa related to gluten ingestion, associated with malabsorption and persisting throughout life in the presence of gluten. The lesion should be shown to improve on a gluten-free diet" (Stewart, 1974). Examination of a jejunal biopsy specimen by stereomicroscopy shows a flat mucosal surface and examination of histologic sections shows crypt hyperplasia and villus atrophy. The initial event is probably an increased shedding of enterocytes which is then compensated for by an increase in the size of the proliferative and maturation compartments of the crypts, coupled with a halving of the cell-cycle time of crypt cells. The net result is a six-fold increase in cell output from the crypts. (Watson and Wright, 1974)

Although the clinical response to a gluten-free diet is usually rapid the histological appearance of the proximal jejunum may not return to normal until two or three years later, or may never entirely return to normal. However, improvements have been shown after six to eight weeks (Yardley et al, 1962).

Malabsorption of water and electrolytes in the proximal jejunum of a patient with coeliac disease was demonstrated by Schedl and Clifton (1963) using a simple double lumen perfusion technique. Schmid et al (1969) using a double lumen tube with occluding balloon, to exclude proximal endogenous secretions from the test segment, demonstrated secretion of water and electrolytes in the jejunum of nine patients with coeliac disease. Three of these patients were perfused again, two to 10 months after introduction of a gluten-free diet, and a secretory state was still found to be present although the patients were in clinical remission.

Reduced absorption, with net secretion in ten cases, was also demonstrated by Russell et al (1972) in a perfusion study of fourteen patients with untreated adult coeliac disease using a triple lumen tube system. A slight improvement in absorption was demonstrated when five of these patients were perfused again two to four months after gluten withdrawal, but the improvement was not marked. The histological appearance of the jejunal mucosa also showed some improvement but none of the biopsies were regarded as entirely normal.

The effects of Vivonex on the morphology of rat jejunum, which have been demonstrated in Chapter 11, showed a reduction in the crypt height to villus height ratio, indicative of improved enterocyte survival. This suggests that Vivonex may have additional advantages for the initial treatment of coeliac disease compared with a normal gluten-free diet. Elemental diet therapy has been reported to be beneficial during the adaptive phase of short gut syndrome in man (Voitk et al, 1973) but has not been investigated in relation to coeliac disease.

Perfusion techniques employing a simple double lumen tube (Schedl and Clifton, 1963) with an infusion point and a distal collection point make no allowance for endogenous secretions entering the test segment from above. Proximal occluding balloons (Phillips and Summerskill, 1966) and the incorporation of a mixing segment in the triple lumen tube system (Cooper et al, 1966) have been used to overcome this inherent error in the study of water and electrolyte absorption in man. The disadvantage of occluding balloons is that they may stimulate peristalsis and cause buckling of the tube in the gut. Sladen and Dawson (1963) compared and simple double-lumen system with a triple-lumen

perfusion system in healthy volunteers and concluded that the magnitude of errors resulting from uncorrected endogenous secretions was negligibly small. In disease states, however, endogenous secretion may be altered and thus a triple-lumen system with a mixing segment will yield more accurate results.

Phenol red and polyethylene glycol with a molecular weight of 4000 (PEG) are commonly used volume markers and have been validated for use in man (Schedl, 1966). The use of PEG was improved by using ^{14}C labelling to simplify its measurement in the perfusates (Wingate et al, 1972) and the need for using carrier PEG has recently been examined (Welman and Barbezat, 1978).

AIM OF STUDY

To determine the effect of supplementing a gluten free diet with Vivonex, for one month, on the absorption of water and electrolytes and on jejunal morphology in patients with untreated adult coeliac disease by comparison with the reported effects of a gluten free diet alone, using a triple lumen tube perfusion system employing phenol red and ^{14}C labelled polyethylene glycol 4000 as non-absorbable markers.

METHODS AND PATIENTS

Patients Studied

Four patients, two male and two female, with untreated adult coeliac disease were studied. They volunteered after a full explanation of the nature of the study. The mean age was 41 years (range 22-66). Of the four patients, two were newly diagnosed cases of adult coeliac disease, one patient had been known to suffer from childhood coeliac disease but had been off a gluten free diet for a period of twelve years and the fourth patient had lapsed from his gluten free diet twelve months previously and had a recurrence

of symptoms. All patients had diarrhoea and this varied from 4-10 motions a day, in one patient steatorrhoea was evident with a faecal fat greater than 30mmol per day, and three patients were mildly anaemic with a haemoglobin range between 12.1-12.9g. All four had low serum folate and one patient had a low serum iron. Jejunal biopsy showed subtotal villus atrophy in three patients and severe partial villus atrophy in the fourth. The diagnosis of coeliac disease was later confirmed by showing improvement of jejunal histology after six months of gluten withdrawal.

Each patient underwent a perfusion and jejunal biopsy at the beginning of the study. They were then commenced on a gluten free diet with Vivonex supplement for a period of one month. Each patient was asked to start with one packet of Vivonex and to increase the daily intake by a packet a day over a period of six days. Three patients were able to take six packets of Vivonex daily (supplying 6 g nitrogen and 1900 calories) without any side effects but the fourth patient only managed to take two packets daily without aggravation of his diarrhoea. The patients were prescribed folic acid in a dose of 5mg 3 hourly.

After one month of treatment with Vivonex and gluten withdrawal the patients underwent a second perfusion and jejunal biopsy.

Methods

A triple lumen tube perfusion technique was used with a 15cm mixing segment and 30cm test segment (Figure 14.1), as previously described (Cooper et al, 1966; Gladen and Dawson, 1968; Russell et al, 1972). Radio-opaque polyvinyl tubing with an internal diameter of 1mm was used (Portex Ltd., Hythe, Kent) and the constituent tubes were cemented together with Portex vinyl cement. A Crosby jejunal biopsy capsule was attached to the end of the tube.

After an overnight fast, the tube was passed orally and positioned under radiographic control so that the infusion point was just distal to the ligament of Treitz. The infusion rate was 15ml/min, which was obtained using a constant flow pump (Watson and Marlow Ltd., Falmouth, Cornwall) and the perfusion fluid consisted of isotonic glucose-saline (56mM glucose) with phenol red (37.5mg/l) and PEG 4000 (1.25mM) labelled with ^{14}C-PEG(5μCi/l) as non-absorbable markers.

The solution was infused for an equilibration period of 60min to obtain steady state conditions (Russell et al, 1972) and then 3 x 10 min collections were made from the proximal and distal collection points using a Watson Marlow pump with a ten channel delta attachment. Standards were taken from the perfusing solution initially and at the end of the perfusion. After the collection had been completed the tube was withdrawan until the biopsy capsule was situated just beyond the ligament of Treitz. The capsule was then fired to obtain a specimen of the jejunal mucosa. The specimen was examined stereomicroscopically and then fixed in formol-saline for histological examination.

The volume of the collections was noted and they were then analysed for glucose and electrolyte concentrations by autoanalyser methods, courtesy of the Department of Pathological Biochemistry at Glasgow Royal Infirmary. Phenol red concentration was determined spectrophotometrically at 560nm, using a Gilford 300N spectrophotometer, by mixing 1ml of sample with 9 ml of trisodium phosphate (0.1N) and mixing well. PEG concentration was determined by adding 10ml of Instagel scintillant to a 0.1ml aliquot of sample and counting on a Packard Tri-Carb liquid scintillation counter (Packard Instruments Ltd., Reading). Quenching was minimal but

was corrected for by automatic external standardisation and prepared standards of ^{14}C.

Calculations

(i) Net water absorption $Q_N = Q_E - Q_L$

$$Q_E = \frac{(I \times Mi)}{(M_p)} - S_p$$

$$Q_L = \frac{Q_E \times M_p}{M_d}$$

Where Q_N = Net flow in or out of segment (-ve indicates secretion)

Q_E = Flow entering segment

Q_L = Flow leaving segment

I = Infusion rate

M_i = Concentration of marker in infused solution

M_p = Concentration of marker in proximal collection

M_d = Marker concentration in distal collection

S_p = Sampling rate proximally

(ii) Net absorption of electrolytes = $E_p \times Q_E - E_d \times Q_L$

Where E_p = Electrolyte concentration in proximal collection

E_d = Electrolyte concentration in distal collection.

Absorption was calculated using both markers and the mean value taken, although similar results were obtained by both methods. The results for each of the three 10 min collections agreed quite closely indicating that steady-state conditions had been obtained and again the mean of the three periods was calculated. Water absorption was expressed as ml $hr^{-1}30cm^{-1}$ and sodium and chloride absorption as mmol $hr^{-1}30cm^{-1}$.

RESULTS

The water, sodium and chloride absorption for each patient, before and after one month of treatment with Vivonex and a gluten-free diet is shown in Table 14.1. The mean water transport before treatment was a net secretion of 61.3 ± 62.3 (± SEM) ml $hr^{-1}30cm^{-1}$ which is comparable to the values reported previously for untreated adult coeliac disease (Russell et al, 1972). After one month of treatment the mean water secretion was 22.5 ± 14.6 ml $hr^{-1}30cm^{-1}$. The sodium and chloride results showed a similar pattern. This slight improvement in mean water absorption is in agreement with the effect of gluten withdrawal without Vivonex supplementation demonstrated previously (Russell et al, 1972) and suggests that Vivonex has conferred no additional advantage to gluten withdrawal alone.

Considering the individual patients, it can be seen that although patient 2 with a severe secretory state showed a marked reduction in secretion, no real change occurred in the other three patients after one month of treatment. All the values are well below those previously reported for normal volunteers (220 ± 21 ml $hr^{-1}30cm^{-1}$). Patient 1 showed a change from net absorption to slight secretion and patient four changed from net secretion to net absorption.

Histological examination of the jejunal biopsies performed at the start of the study revealed subtotal villus atrophy in three patients and severe partial villus atrophy in the fourth. After one month of treatment repeat jejunal biopsies revealed only minor changes in the histological appearances. One case had deteriorated and showed subepithelial deposition of collagen. The other three cases showed some mild improvement with a reduction of the plasma

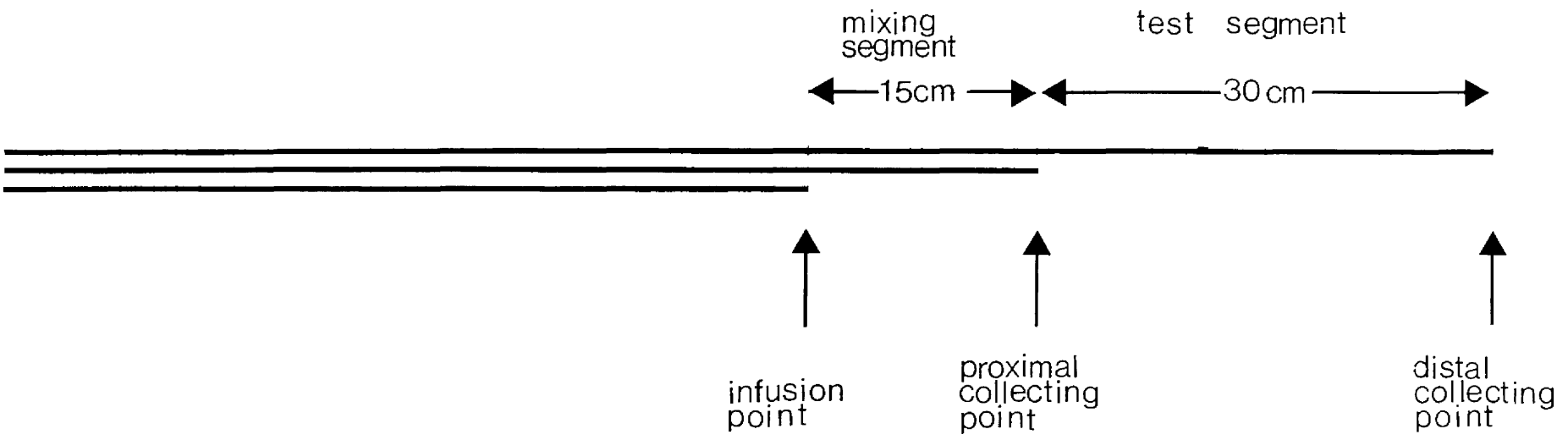
cell infiltrate and improvement in the height of the surface epithelium. Clinical improvement occurred in all 4 patients during the test period; they felt generally better and their diarrhoea disappeared.

DISCUSSION

This study failed to show any marked improvement in the absorption of water and electrolytes across the jejunal mucosa in patients with adult coeliac disease after one month of treatment with Vivonex and gluten withdrawal. These findings are similar to those reported for a gluten-free diet alone (Russell et al, 1972). The histological changes indicate that only minimal improvement occurred, no greater than would have been expected from a gluten free diet alone (Yardley et al, 1962). The degree of clinical improvement that occurred cannot be attributed solely to the elemental diet for such an improvement can be accounted for simply by gluten withdrawal.

In the majority of cases of coeliac disease, treatment with a gluten free diet induces clinical remission, and over a longer period of time histological improvement of the proximal jejunum occurs (Stewart, 1974). The failure of Vivonex to promote a more rapid recovery of function than a gluten free diet alone is perhaps surprising in view of its effects on normal rat jejunum. However, since the lack of residue in the elemental diet is perhaps its most important property, it may be that Vivonex alone would have resulted in a greater improvement. The unpalatable nature of Vivonex presents problems of patient acceptance which are greater when the elemental diet is the only nutrition allowed, and supplementing the elemental diet with normal gluten-free food provides additional nutritional support.

FIGURE 14.1. DIAGRAM OF THE TRIPLE LUMEN TUBE USED FOR THE PERFUSION STUDY IN COELIAC DISEASE.


mixing segment
test segment
15cm
30 cm
infusion point
proximal collecting point
distal collecting point

Patient Number	BEFORE STUDY			AFTER ONE MONTH		
	Water Transport ml h^{-1} $30cm^{-1}$	Sodium Transport m.mol h^{-1} $30cm^{-1}$	Chloride Transport m.mol h^{-1} $30cm^{-1}$	Water Transport ml h^{-1}	Sodium Transport m.mol h^{-1}	Chloride Transpo m.mol h^{-1} $30cm^{-1}$
1	42	1.92	2.76	-3.9	-3.9	-4.4
2	-242	-30.4	-32.8	-43.5	-9.5	-10.8
3	- 33	- 5.7	- 8.8	-51	-10.8	- 9.3
4	- 12	- 1.2		8.6	2.4	0.6
Mean ±SEM	-61.3 ± 62.3	-8.9 ± 7.4	-12.9 ± 10.5	-22.5 ± 14.6	-5.45 ± 3.0	-5.9 ± 2.6

TABLE 14.1 THE ABSORPTION OF WATER AND ELECTROLYTES ACROSS THE JEJUNAL MUCOSA BEFORE ELEMENTAL DIET THERAPY AND AFTER ONE MONTH OF TREATMENT

SECTION IV

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION

The work reported in this thesis examines the effect of commercially available 'elemental' diets on bile acid metabolism and small bowel structure and function, in rats and man. Much of the work is animal studies, but these have been extended to humans and the role of Vivonex in the management of bile acid induced diarrhoea was investigated. Pancreatic secretion was also studied in man, and the reduced trypsin secretion found with Vivonex, compared with the Lundh test meal, indicated a reduced cholecystokinin-pancreozymin response. This suggests that gallbladder contraction may also be decreased during Vivonex intake. The changes in jejunal morphology induced by elemental diet feeding in rats led to the investigation of the effects of Vivonex in patients with untreated adult coeliac disease.

Bile acid metabolism and small intestinal structure and function are intimately related: deconjugated bile acids may damage the small intestine; pancreatic and biliary secretions may have an important role in maintaining normal small bowel structure and function; altered small intestinal function may affect bile acid absorption.

Feeding deoxycholic acid damages the small intestine of mice (Fry and Staffeldt, 1964) and rats (Gracey et al, 1973). Mucosal hypertrophy with an increased crypt height to villus height ratio, and a marked increase in the ratio of free to conjugated bile acids, was demonstrated in self-filling blind loops of rat intestine (Bloch et al, 1975) and similar mucosal injury was demonstrated by Hoskes and co-workers using the same rat model (Hoskes et al, 1975).

The importance of the small amount of deconjugated bile acids which may normally be present in the small intestine in relation to structure and function is unknown, but the ratio of free to conjugated bile acids in rat intestine was found to be 1 : 11 (Bloch et al, 1975) and deconjugated bile acids have been demonstrated as a normal occurrence in the lower jejunum in man (Northfield and McColl, 1973). The total amount of deconjugation may be greater than measurements of concentration suggest, since passive absorption of unconjugated bile acids in the upper small intestine is much greater than that of the conjugates (Schiff et al 1972).

The influence of elemental diet feeding on deconjugation of bile acids has not been investigated in this study, but if the bacterial flora is altered, as suggested by the reduced dehydroxylation of bile acids in the Vivonex fed rats, then decreased deconjugation of bile salts may contribute to the reduced CH:VH ratio observed. A similar situation exists in the germ-free rat where bile acid deconjugation (Kellogg et al, 1970) and the CH:VH ratio (Galjaard et al, 1972) are both reduced in comparison with conventional rats.

Studies in rats with total biliary diversion suggest that bile acids inhibit cell proliferation and migration in the jejunum (Reicht et al, 1975) and low concentrations of deconjugated bile acids inhibit transport of solutes and the intermediate metabolism of the intestinal mucosa (Dietschy, 1967). Trophic effects of pancreatic secretions, and to some extent biliary secretions, on the intestinal mucosa have also been demonstrated, as discussed in Chapters 10, 11 and 13, and enhanced bile acid absorption has been suggested as the mechansism of the reduced bile acid turnover

observed in germ-free rats (Hellogg and Wostmann, 1969). Thus bile acid metabolism, together with pancreatic function, is closely related to small intestinal structure and function.

The observed effects of elemental diet feeding have been considered in relation to their special properties and composition compared with normal solid diets. The elemental diets contain no indigestible residue or bulk; the carbohydrate source is simple sugars, consisting of oligosaccharides, with an average chain length of five monosaccharide units, in addition to glucose or sucrose; nitrogen is supplied as synthetic amino acids in Vivonex, and amino acids plus small peptides from hydrolysed casein in Flexical; Vivonex is virtually fat-free but contains some purified Safflower oil to supply essential fatty acids and the higher fat content of Flexical includes medium chain triglycerides which are more readily absorbed than the long chain ones.

The results obtained for the effect of elemental diet feeding in rats on total faecal bile acid excretion (Chapter 6) suggest that the low residue nature of Vivonex may be its most important property in this respect, rather than the low fat content, since a similar reduction in faecal bile acid excretion was found with Flexical which does contain a significant amount of fat.

When the composition of the bile acids excreted was examined (Chapter 7), the degree of bacterial degredation was found to be reduced by Vivonex feeding. The absolute amounts of the secondary bile acid peaks, lithocholic and deoxycholic acid, were significantly reduced and the proportion of these two bile acids, as a percentage of the total excreted, was also significantly reduced. The absolute amountof the cholic acid peak excreted

was not significantly reduced, and the relative proportion of this peak did in fact increase, during Vivonex feeding. Thus not only the amount of bacterial degredation products, but also their relative proportion, was reduced during Vivonex feeding and this indicates that the bacterial population may be reduced or altered in the caecum and colon. In contrast, the absolute amount of the cholic acid peak excreted was significantly reduced during Flexical feeding and the proportion was decreased, although not significantly. This suggests that the low fat content of Vivonex may be an important factor since this is the main difference between the two diets.

The measurement of cholic acid half-life in Chapter 3 also revealed possible differences between the two elemental diets. The prolonged half-life induced by Flexical feeding is comparable to that reported previously for semi-synthetic diet feeding in normal rats. Vivonex feeding, however, resulted in an even longer half-life of cholic acid which is comparable to that reported for germ-free rats fed semi-synthetic diets, or that of normal rats treated with antibiotics. The results obtained are in agreement with the reports in the literature that liquid, semi-synthetic diets reduce bile acid turnover, but the bacterial population of the gut may be altered and have an additional effect during Vivonex feeding. The results for bile acid composition and turnover therefore suggest that Vivonex has a greater effect than Flexical, which may be mediated by alteration of the bacterial population of the gut, and result from the difference in fat content of the two diets.

Cholesterol accumulation in the liver was also greater during Vivonex feeding than Flexical feeding and this probably

reflects the difference in cholic acid half-life. A greater reduction in bile acid turnover means that less cholesterol is being excreted as bile acids and more may accumulate in the liver unless a compensation occurs in the rate of synthesis.

The importance of bacterial changes in the observed alterations of bile acid metabolism during elemental diet feeding could best be assessed by repeating the experiments in germ-free animals. Alas, no access to a germ-free rat colony was available. The use of antibiotics would provide an alternative, however, and the role of fibre in the diet could be elucidated by feeding the elemental diets with added cellulose, pectin or other suitable non-digestible residue.

The reduction in faecal bile acid excretion in rats induced by Vivonex feeding prompted the investigation of the use of the diet in the management of patients with cholerheic diarrhoea (Chapter 9). In these patients malabsorption of bile acids, as a result of resection of the terminal ileum leads to an excessive load of bile acids entering the colon, where they cause secretion of water and electrolytes. Vivonex therapy reduced the bile acid excretion in the patients studied with a concomittant improvement in their diarrhoea. The low residue/nature of the diet may be partly responsible for this effect. Lack of residue will reduce bile acid absorption in the small bowel and prolong transit time, thus enhancing bile acid absorption. The low fat content of Vivonex might also contribute, in man, by reducing gallbladder stimulation and hence the entry of bile acids into the duodenum.

Trypsin secretion in response to Vivonex ingestion was less than with the Lundh test meal stimulus (Chapter 10), indicating a reduced cholecystokinin-pancreozymin response compatible with reduced

gallbladder stimulation. This response may be mediated by the low nitrogen and high glucose content of Vivonex in addition to its low fat content, however. Bile acids and trypsin were shown to be decreased in ileal fistula drainage during administration of Vivonex (Hill et al, 1976), which also suggests that bile acid secretion into the duodenum is reduced. A controlled comparison with Flexical would elucidate the importance of the low fat content, but this has not been done since the unpalatable nature of the diets makes it difficult to persuade patients to consume them.

No indication of whether bacterial alterations are involved in the reduction of faecal bile acids in man was obtained. It is mainly primary bile acids that are excreted by patients with ileal resection anyway, and thus reduced bacterial degredation cannot be detected from an altered faecal bile acid composition.

The morphological changes in the small intestine of rats induced by elemental diet feeding (Chapter 11) also resemble those of germ-free rats and so once again alteration of the bacterial population of the small bowel may be implicated. The changes observed indicate an improved survival of the mature enterocyte population and the low residue nature of the elemental diets would contribute to this by reducing the physical stress on the villus cells. The fact that a reduction of the CH to VH ratio also occurred in the ileum of the rats fed the elemental diets suggests a remote hormonal or neurovascular mediation. The rapidly absorbable nature of the diets may affect the status of this postulated regulatory mechanism. The evidence for the existence of such a mechanism in the control of gut structure and function has been discussed in Chapters 11 and 15. It has also been

demonstrated that proximal enterectomy results in a greater hyperplastic response in the ileum than jejunal bypass of pancreaticobiliary diversion, and suggests that factors other than increased luminal nutrition are involved in the adaptive response of the ileum (Williamson et al, 1973).

The reduction in CH to VH ratio observed in rats led to the study of the use of Vivonex in untreated adult coeliac disease, reported in Chapter 14. This study can be criticised in that a gluten-free diet, in addition to Vivonex, was used and not the elemental diet alone. If the low residue nature of the diet is an important property then administration of Vivonex alone might result in a more marked improvement in the structure and function of the jejunum. Also the proximal jejunum, which was the segment perfused in this study, is the most badly damaged part of the small intestine in coeliac disease and it is possible that there was a greater improvement in function in the distal jejunum than would have been produced by a gluten free diet alone.

There are problems with patient acceptance of the unpalatable elemental diets, and since clinical remission occurs rapidly following the introduction of a gluten free diet in coeliac disease, it was not felt that treatment with Vivonex alone could be justified. In cholerheic diarrhoea no proven dietary treatment is available and so Vivonex alone was used in these patients. A rat model for coeliac disease using Trinarenol administration has been proposed (Robinson, 1972). It is not an entirely satisfactory model but it would be interesting to use it to investigate the effect of Vivonex on the recovery of the small intestinal mucosa by studying both its morphological aspects and also the water and glucose absorption in vivo.

Water and glucose absorption were not enhanced in the jejunum of the normal rats fed Vivonex when measured by perfusion in vivo, and were in fact reduced in the rats fed Flexical compared with the controls (Chapter 13). This difference between the two elemental diets may result from differences in the nature of the carbohydrate and nitrogen sources in the diet alterating the hormonal or neurovascular regulation of gut function in different ways. As discussed in Chapter 13, the nutritional requirements of the animal may also regulate absorption in vivo.

Since this work was completed, Gardner and Heading have published evidence that Vivonex enhances water and glucose absorption by the entire small intestine in vitro (Gardner and Heading, 1979). Their study is not strictly comparable as an in vitro perfusion technique was used, which eliminates any effects of vascular regulations of absorption, and also the entire jejunum plus ileum was perfused. As suggested previously (Chapter 13), the ileum may be more susceptible to an increase in absorptive capacity than the jejunum and this will be investigated by repeating the experiment, and perfusing the ileum in vivo.

CONCLUSIONS

It has been demonstrated that elemental diets cause a profound alteration of bile acid metabolism in rats, with a reduction of faecal bile acid excretion, and this has been successfully applied in the management of patients with cholerheic diarrhoea. Pancreatic stimulation is also reduced to some extent by Vivonex, and the diet may be a useful alternative to normal oral feeding in patients with pancreatic insufficiency unsuitable for intravenous feeding.

Small bowel structure after elemental diet feeding in rats showed morphological changes indicative of improved enterocyte survival, both in the ileum and jejunum, but these changes were not accompanied by a demonstrable improvement in jejunal function. No obvious benefit was found in patients with adult coeliac disease treated with a gluten-free diet plus Vivonex, but Vivonex alone might perhaps have conferred an advantage over a gluten-free diet. The recovery of Trinaranol damaged rat intestinal mucosa during elemental diet feeding should be investigated, and then if the animal studies are encouraging the study in coeliac disease could be repeated using Vivonex therapy alone.

The low residue nature of Vivonex and Flexical, together with the rapidly absorbed nature of the nutritional source, may be their important properties. The importance of the low-fat nature of Vivonex needs further elucidation by direct comparison with Flexical in human studies, for example of the trypsin response, but it does not appear to be the most important property. Many of the effects of the diets in the longer term animal studies reproduce the situation in germ-free animals, and so it is possible that the effects are at least partly mediated by modification of the bacterial flora.

REFERENCES

Abderhalden, E., Frank, F. and Schittenhelm, A. (1909)
On the utilization of completely hydrolysed protein in the human organism.
Hoppe-Seyler's Zeitschrift für Physiologische Chemie, 63, 215

Abderhalden, E. and Hirsch, P. (1912)
Feeding experiments with gelatin, ammonium salts, completely hydrolysed meat and a mixture of all known amino acids on young dogs.
Hoppe-Seyler's Zeitschrift für Physiologische Chemie, 81, 323

Abell, L.L., Levy, B.B., Brodie, B.B. and Kendall, F.E. (1952)
A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity.
Journal of Biological Chemistry, 195, 357-366

Abrams, G.D., Bauer, H. and Spring, H. (1963)
Influence of the normal flora on mucosal morphology and cellular renewal in the ileum.
Laboratory Investigation, 12, 355-364

Adams, W.R., Copeland, E.M., Dudrick, S. J., Johnson, L.R. and Castro, J.A. (1973)
Maintenance of gut mass in bypassed bowel of orally versus parenterally nourished rats.
Journal of Surgical Research, 24, 421-427

Adibi, S.A. (1971)
Intestinal transport of dipeptides in man: relative importance of hydrolysis and intact absorption.
Journal of Clinical Investigation, 50, 2266-2275

Adibi, S.A., Fogel, M.R. and Agrawal, R.M. (1974)
Comparison of free amino acid and dipeptide absorption in the jejunum of sprue patients.
Gastroenterology, 67, 586-591

Allan, J.G., Gerskowitch, V.P., and Russell, R.I. (1974)
The role of bile acids in the pathogenesis of postvagotomy diarrhoea.
British Journal of Surgery, 61, 516-518

Alpers, D.H., and Solin, M. (1970)
The characterization of rat intestinal amylase.
Gastroenterology, 58, 335-342

Altmann, G.G. (1971)
Influence of bile and pancreatic secretions on the size of the intestinal villi in the rat.
American Journal of Anatomy, 132, 167-178

Altmann, G. G., and Leblond, C.P. (1967)
Cell number as a measure of distribution and renewal of epithelial cells in the small intestine of growing and adult rats.
American Journal of Anatomy, 121, 319-336

Antonis, A. and Bersohn, I. (1962)
The influence of diet on faecal lipids in South African white and Bantu prisoners.
American Journal of Clinical Nutrition, 11, 142-155

Attebury, H. R., Sutter, V.L. and Finegold, S.M. (1972)
Effect of a partially chemically defined diet on normal human faecal flora.
American Journal of Clinical Nutrition, 25, 1391-1398

Austad, W.I., Lack, L. and Tyor, M.P. (1967)
Importance of bile acids and an intact distal small intestine for fat absorption.
Gastroenterology, 52, 638-646

Axelsson, C.K. and Justesen, T. (1977)
Studies on the duodenal and faecal flora in gastrointestinal disorders during treatment with an elemental diet.
Gastroenterology, 72, 397-401

Baker, R. D. and Searle, G. W. (1960)
Bile salt absorption at various levels of rat small intestine
Proceedings of the Society for Experimental Biology and Medicine
105, 521-523

Batt, R.M. and Peters, T.J. (1976)
Absorption of galactose by the rat intestine in vivo: proximal distal kinetic gradients and a new method to express absorption per enterocyte.
Clinical Science and Molecular Medicine, 50, 499-507

Beher, W. T., Filus, A.M. Rao, B and Beher, M.L. (1969)
A comparative study of bile acid metabolism in the rat, mouse, hamster and gerbil.
Proceedings of the Society for Experimental Biology and Medicine
130, 1067-1071

Bergmeyer, H.U. (1963)
Methods of Enzymatic Analysis pp 733-735
Weinheim: Verlag Chemie

Bergström, S. (1962)
Metabolism of bile acids
Federation Proceedings, 21 (Suppl. 11), 23-32

Bergström, S., Danielsson, H. and Samuelsson, B. (1960)
Formation and metabolism of bile acids.
In Lipide Metabolism, ed. Bloom, K. pp 291-336
New York: Wiley

Birnbaum, S. M. Greenstein, J.P., and Winitz, M. (1957a)
Quantitative nutritional studies with water soluble chemically defined diets. II Nitrogen balance and metabolism.
Archives of Biochemistry and Biophysics, 72, 417-427

Birnbaum, S.M., Winitz, M. and Greenstein, J.P. (1957b)
Quantitative nutritional studies with water soluble chemically defined diets. III Individual amino acids as sources of non-essential nitrogen.
Archives of Biochemistry and Biophysics, 72, 423-436

Bloch, R., Menge, H., Lorenz-Meyer, d., Stöckert, H.G. and Reicken, E.C. (1975)
Functional, biochemical and morphological alterations in the intestines of rat with experimental blind-loop syndrome.
Research in Experimental Medicine, 166, 67-73

Bolin, T.D., McKern, A. and Davis, A.E. (1971)
The effect of fat on lactose activity in the rat.
Gastroenterology, 60, 432-437

Borgström, B. (1953)
On the mechanism of the intestinal fat absorption. The effect of bile diversion on fat absorption in the rat.
Acta Physiologica Scandinavica, 28, 279-286

Borgström, B., Dahlqvist, A., Lundh, G and Sjövall, J. (1957)
Studies of intestinal digestion and absorption in the human.
Journal of Clinical Investigation, 36, 1521-1536

Borgstrom, B., Lundh, G.and Hofmann, A.F. (1963)
The site of absorption of conjugated bile acids in man.
Gastroenterology, 45, 229-238

Bounous, G., Cronin, R.F. and Gurd, F.N. (1967a)
Dietary prevention of experimental shock lesions
Archives of Surgery, 94, 46-60

Bounous, G., Sutherland, D.G., McArdle, A. H. and Gurd, F.N. (1967b)
The prophylactic use of an elemental diet in experimental haemorrhagic shock and intestinal ischaemia.
Annals of Surgery, 166, 512-522

Bounous, G., Hugon, J.S. and Gentile, J.M. (1971a)
Elemental diet in the management of the intestinal lesion produced by 5-fluorouracil in the rat.
Canadian Journal of Surgery, 14, 298-311

Bounous, G., Gentile, J.M. and Hugon, J.S. (1971b)
Elemental diet in the management of the intestinal lesion produced by 5-fluorouracil in man.
Canadian Journal of Surgery, 14, 512-524

Bounous, G., Devroede, G., Haddad, H., Beudray, R., Perey, B.J. and Lejune, L.P. (1974)
Use of an elemental diet for intestinal disorders and for the critically ill.
Diseases of the Colon and Rectum, 17, 157-165

Bounous, G. and Devroede, G.J. (1974)
Effects of an elemental diet on normal human faecal flora.
Gastroenterology, 66, 210-214

Bronk, J.R. and Ingram, P.A. (1976)
Sugar transfer during vascular perfusion of rat jejunum.
Journal of Physiology, 226, 36-37.

Bronk, J.R. and Leese, M.J. (1974)
The absorption of a mixture of amino acids by rat small intestine
Journal of Physiology, 241, 271-286

Brown, R.A., Thompson, A.G., McArdle, A.H. and Gurd, F.N. (1970)
Alteration of exocrine pancreatic storage enzymes by feeding an elemental diet: a biochemical and ultrastructural study.
Surgical Forum, 21, 391-393

Bury, K.D. and Jambunathan, G. (1974)
Effects of elemental diets on gastric emptying and gastric secretion in man.
American Journal of Surgery, 127, 59-64

Bury, K.D., Stephens, R.V. and Randall, H.T. (1971)
Use of a chemically defined liquid elemental diet for nutritional management of fistulas of the alimentary tract
American Journal of Surgery, 121, 174-183

Campbell, C.B., Cowley, D.J. and Dowling, R.H. (1972)
Dietary factors affecting biliary lipid secretion in the Rhesus monkey.
European Journal of Clinical Investigation, 2, 332-341

Campbell, A.H., Sewell, W.R., Chudkowski, M., Willson, J.E., Lord, G.H. and Mohamed, K. (1973)
The effects of feeding an elemental chemical diet to mature rats: toxicologic and pathologic studies.
Toxicology and Applied Pharmacology, 26, 63-71

Carey, M.C. and Small, D.M. (1972)
Micelle formation by bile salts.
Archives of Internal Medicine, 130, 506-527

Cheng, H. and Leblond, C.P. (1974)
Origin, differentiation and renewal of the four main cell types in the mouse small intestine. V Unitarian theory of the origin of the four main cell types.
American Journal of Anatomy, 141, 537-562

Colton, T. (1974)
Statistics in Medicine p 213 Boston: Little,Brown & Co.

Cook, H.B., Lennard-Jones, J.E., Sherif, S.M. and Wiggins, H. (1967)
Measurement of tryptic activity in intestinal juice as a diagnostic test of pancreatic disease.
Gut, 8, 408-414

Cooper, K., Levitan, R., Fordtran, J.S. and Ingelfinger, F.J. (1966)
A method for studying absorption of water and solute from the human small intestine
Gastroenterology, 50, 1-7

Cough, K.B., Watkin, D.M., Smith, R.R., Rosenberg, L.E., Winitz, M., Birnbaum, S.M., Otey, M.C., and Greenstein, J.P. (1960)
Clinical tests of water soluble chemically defined diets.
Federation Proceedings, 19, 13 (Abstract)

Coupar, I.M. and McColl, I. (1975)
Stimulation of water and sodium secretion and inhibition of glucose absorption from the rat jejunum during intra-arterial perfusions of prostaglandins.
Gut, 16, 759-765

Crampton, R.F., Gangolli, S.D., Simson, P. and Matthews, D.M. (1971)
Rates of absorption by rat intestine of pancreatic hydrolysates of protein and there corresponding amino acid mixtures.
Clinical Science, 41, 409-417

Crane, R.K. (1965)
Sodium dependant transport into the intestine and other animal tissues.
Federation Proceedings, 24, 1000-1006

Creamer, B. (1974)
The small intestine vol-23. London:Heinemann

Crowther, J.S., Drasar, B.S., Goddard, P., Hill, M. J. and Johnson, K. (1975)
The effect of a chemically defined diet on the faecal flora and faecal steroid concentration.
Gut, 14, 790-795

Cummings, J.H. (1973)
Dietary fibre.
Gut, 14, 69-81

Cummings J.H., James, W.P.T. and Wiggins, H.S. (1975)
Role of the colon in ileal resection diarrhoea.
Lancet, 1, 344-347

Dahlqvist, A. (1964)
Method for assay of intestinal disaccharidases.
Analytical Biochemistry, 7, 18-25

Dahlqvist, A. and Borgström, B. (1961)
Digestion and absorption of disaccharides in man.
Biochemical Journal, 81, 411-418

Dahlqvist, A., Hull, S. and Gustafsson, B.E. (1965)
Rat intestinal 6-bromo-2naphtyl-glycosidase and disaccharidase activities. I Enzymic properties and distribution in digestive tract of conventional and germ-free animals.
Archives of Biochemistry and Biophysics, 109, 150-153

Danielsson, H. (1963)
Present status of research on catabolism and excretion of cholesterol.
In Advances in Lipid Research, Eds. Paoletti, R. and Kritchevsky, D. Vol 1, pp295-335
New York:Academic Press

Davidson, R.J. and Leese, H.J. (1977)
Sucrose absorption by rat small intestine in vivo and in vitro.
Journal of Physiology, 267, 237-248

Dawson, A.M. (1967)
Absorption of fats.
British Medical Bulletin, 23, 247-251

Debnam, E.S. and Levin, R.J. (1975)
An experimental method of identifying and quantifying the active transfer electrogenic component from the diffusive component during sugar absorption measured in vivo.
Journal of Physiology, 246, 181-196

Demarne, Y., Jacquet, J., Heijenoort, Y. and Mathis, J. (1974)
Influence d'une supplementation de la ration en sels biliaires conjugués sur l'equipement en acides biliaires de l'intestin grêle du rat.
Annals de Biologie Animale, Biochimie, Biophysique, 14, 239-250

Deren, J.J., Broitman, S.A. and Zamcheck, N. (1967)
Effect of diet upon intestinal disaccharidases and disaccharide absorption.
Journal of Clinical Investigation, 46, 186-195

Devereux, M.F. and Baker, A.R. (1977)
The effect of dietary fibre on stool bulk and gut transit time
Gastroenterology, 72, 1043 (Abstract)

Dietschy, J.M. (1967)
Effects of bile salts on intermediate metabolism of the intestinal mucosa.
Federation Proceedings, 26, 1539-1543

Dietschy, J.M. (1968)
Mechanisms for the intestinal absorption of bile acids.
Journal of Lipid Research, 9, 297-309

Dowling, R.H. (1972)
The enterohepatic circulation.
Gastroenterology, 62, 122-140

Dowling, R.H. and Booth, J.J. (1967)
Structural and functional changes following small intestinal resection in the rat.
Clinical Science, 32, 139-149

Bowling, R.N., Mack, E. and Small, D.M. (1970)
Effects of controlled interruption of the enterohepatic circulation of bile salts by biliary diversion and by ileal resection on bile salt secretion, synthesis and pool size in the rhesus monkey.
Journal of Clinical Investigation, 49, 232-242

Bowling, R.N., Reicken, E.O., Laws, J.W. and Booth, C.C. (1967)
The intestinal response to high bulk feeding.
Clinical Science, 32, 1-9

Dworkin, L.D., Levine, G.M., Farber, M.J. and Spector, M.H. (1976)
Small intestinal mass of the rat is partially determined by indirect effects of luminal nutrition.
Gastroenterology, 71, 626-630

Dyck, W.P. (1971)
Influence of intrajejunal glucose on pancreatic exocrine function in man.
Gastroenterology, 60, 364-369

Eastwood, G.L. (1977)
Gastrointestinal epithelial cell renewal.
Gastroenterology, 72, 962-975

Eastwood, M.A., and Boyd, G.S. (1967)
The distribution of bile salts along the small intestine of rats.
Biochimica et Biophysica Acta, 137, 393-396

Eastwood, M.A., Kirkpatrick, J.R., Mitchell, W.D., Bone, A. and Hamilton, T. (1973)
Effects of dietary supplements of wheat bran and cellulose on faeces and bowel function.
British Medical Journal, 4, 392-394

Eckhauer, F., Feyerabend, G., and Raffler, H. (1973)
Analysis of the effects of food and digestive secretion on the small intestine of the rat. III Mucosal mass, activity of brush border enzymes and in vivo absorption of galactose, sodium and potassium.
Gut, 19, 707-714

Eckhauer, F.E.K., Siegar, R., Lichtenberger, L.M. and Johnson, L.R. (1973b)
Role of food-bulk in maintaining mucosal morphology of rat small intestine.
Gastroenterology, 74, 1080 (Abstract)

Eichholz, A. and Crane, R.K. (1965)
Studies on the organisation of the brush border in intestinal epithelial cells. I Preparation of isolated hamster brush borders and density gradient separation of fractions.
Journal of Cell Biology, 26, 687-691

Einarsson, K. (1966)
On the formation of hyodeoxycholic acid in the rat.
Journal of Biological Chemistry, 241, 534-539

Lertran, A., Brooks, F.P., Ostrow, J.D., Aryan, D.A., Williams, H. and Jerla, J.J. (1971)
Effect of jejunal amino acid perfusion and exogenous cholecystokinin on the exocrine pancreatic and biliary secretions in man.
Gastroenterology, 61, 686-692

Mrari, E. and Janssen, G. (1968)
Gas-liquid chromatographic determination of human faecal bile acids.
Journal of Lipid Research, 9, 226-236

Feldman, E.J., Dowling, R.H., McNaughton, J. and Peters, T.J. (1976) Effects of oral versus intravenous nutrition on intestinal adaptation after small bowel resection in the dog.
Gastroenterology, 70, 712-719

Ferguson, A., Gerskowitch, V.P. and Russell, R.I. (1973)
Pre and post weaning disaccharidase patterns in isografts of foetal mouse intestine.
Gastroenterology, 64, 202-207

Fisher, R.B. and Gardner, M.L.G. (1974)
A kinetic approach to the study of absorption of solutes by isolated perfused small intestine.
Journal of Physiology, 241, 211-234

Fisher, R.B. and Gardner, M.L.G. (1976)
A diurnal rhythm in the absorption of glucose and water by isolated rat small intestine.
Journal of Physiology, 254, 821-825

Fogel, M.R. and Gray, G.M. (1970)
Carbohydrate digestion and absorption.
Gastroenterology, 58, 96-107

Fordtran, J.S. and Locklear, T.W. (1966)
Ionic constituents and osmolality of gastric and small intestinal fluids after eating.
American Journal of Digestive Diseases, 11, 503-521

Fordtran, J.S., Rector, F.C. and Carter, N.W. (1968)
The mechanism of sodium absorption in the human small intestine.
Journal of Clinical Investigation, 47, 884-900

Fry, A.J.L. and Staffeldt, E. (1964)
Effect of a diet containing sodium deoxycholate on the intestinal mucosa of the mouse.
Nature, 203, 1396-1398

Galjaard, H., van der Meer-Fiennes, W. and Giesen, J. (1972)
Feedback control by functional villus cells on cell production and maturation in intestinal epithelium.
Experimental Cell Research, 73, 197-207

Gall, J.L., Fenton, P.F. and Cowgill, G.R. (1943)
The nutrition of the mouse. II Effect of diet on the bacterial flora of the intestine and the caecum.
Journal of Nutrition, 35, 13-25

Gardner, M.L.G. and Reading, R.J. (1979)
Effects of elemental diets on absorptive and enzymic activities and on 5-fluorouracil toxicity in rat small intestine.
Clinical Science, 56, 243-249

Gardner, M.L.G., Benson, A.R. and Reading, R.J. (1978)
Changes in absorptive and peptide hydrolase activities in rat small intestine after administration of 5-flourouracil.
Clinical Science and Molecular Medicine, 54, 411-418

Gleeson, M.H., Bullen, J. and Bowling, R.W. (1972)
Intestinal structure and function after small bowel bypass in the rat.
Clinical Science, 43, 731-742

Go, V.L.W., Hofmann, A.F. and Summerskill, W.H.J. (1970)
Pancreozymin bio-assay in man based on pancreatic enzyme secretion:potency of specific amino acids and other digestive products.
Journal of Clinical Investigation, 49, 1558-1564

Goode, A., Hawkins, M., Feggetter, J.G.W. and Johnston, I.D.A. (1975) Use of an elemental diet for long-term nutritional support in Crohn's disease.
Lancet, 1, 122-124

Gorbach, S.L., Plaut, A.G., Nahas, L. and Weinstein, L. (1967)
Studies on intestinal microflora. II Microorganisms of the small intestine and their relations to oral and faecal flora.
Gastroenterology, 53, 356-367

Gordon, H., Lewis, B., Eales, L. and Broch, J.F. (1957
Dietary fats and cholesterol metabolism. Faecal elimination of bile acids and other lipids.
Lancet, 2, 1299-1306

Gracey, M., Papadimitriou, J., Burke, V., Thomas, J. and Bower, G. (1975)
Effects on small intestinal function and structure induced by feeding a deconjugated bile salt.
Gut, 14, 519-523

Greenstein, J.P., Birnbaum, S.M., Winitz, M. and Otey, M.C. (1957)
Quantitative nutritional studies with water soluble, chemically defined diets. I Growth, reproduction and lactation in rats.
Archives of Biochemistry and Biophysics, 72, 396-416

Greenstein, J.P., Otey, M.C., Birnbaum, S.M. and Winitz, M. (1960)
Quantitative nutritional studies with water soluble chemically defined diets. X Formulation of a nutritionally complete liquid diet.
Journal of National Cancer Institute, 24, 211-219

Greenstein, J.P. and Winitz, M. (1961)
Amino acids in nutrition.
In Chemistry of the Amino Acids, Ch. 3. New York:Wiley

Grundy, S.M., Ahrens, E.H. and Miettenen, T.A. (1965)
Quantitative isolation and gas-liquid chromatographic analysis of total fecal bile acids.
Journal of Lipid Research, 6, 397-409

Gustafsson, B.E. and Norman, A. (1969a)
Influence of diet on the turnover of bile acids in germ-free and conventional rats.
British Journal of Nutrition, 23, 429-442

Gustafsson, B.E. and Norman, A. (1969b)
Influence of diet on the composition of faecal bile acids in rats.
British Journal of Nutrition, 23, 627-635

Gustafsson, B.E., Norman, A. and Sjövall, J. (1960)
Influence of E.coli infection on turnover and metabolism of cholic acid in germ-free rats.
Archives of Biochemistry and Biophysics, 91, 93-100

Harvey, R.F., Pomare, E.W. and Heaton, K.W. (1973)
Effects of increased dietary fibre on intestinal transit.
Lancet, 1, 1273-1280

Helman, J.A. and Barbezat, G.O. (1973)
Comparison of ^{51}Cr labelled polyethylene glycol (PEG) with carrier PEG and ^{14}C-PEG alone as volume indicator in the human jejunum.
Gut, 13, 155-156

Hepner, G.W. (1975)
Effect of decreased gallbladder stimulation on enterohepatic cycling and kinetics of bile acids.
Gastroenterology, 68, 1574-1581

Herrman, R.G. (1957)
Determination of serum and tissue cholesterol.
Proceedings of the Society for Experimental Biology and Medicine, 94, 503-512

Hietanen, E. and Hänninen, O. (1971)
Comparison of mucosal metabolism in the small intestine of specific pathogen-free and conventional rats.
Scandinavian Journal of Gastroenterology, 6, 127-131

Hill, M.J. (1971)
The effect of some factors on the faecal concentration of steroids, neutral steroids and urobilins.
Journal of Pathology, 104, 239-245

Hill, M.J. and Drasar, B.S. (1968)
Degradation of bile acids by human intestinal bacteria.
Gut, 9, 22-27

Hill, G.L., Blair, S.J., Edwards, J.P. and Goligher, J.C.(1976)
Decreased trypsin and bile acids in ileal fistula drainage during the administration of a chemically defined liquid elemental diet.
British Journal of Surgery, 63, 133-136

Ho, K.J. (1976)
Circadian distribution of bile acids in the enterohepatic circulatory system in rats.
American Journal of Physiology, 230, 1331-1335

Hofmann, A. F. (1964)
Thin layer chromatography of the bile acids and their derivatives
In New biochemical separations. ed. James, A.T. and Morris, L.J.
pp 262-282. Princeton:van Nostrand

Hofmann, A.F. (1967)
The syndrome of ileal disease and the broken enterohepatic circulation: cholerheic enteropathy.
Gastroenterology, 52, 752-757

Hofmann, A.F. (1972)
Bile acid malabsorption caused by ileal resection.
Archives of Internal Medicine, 130, 597-605

Hofmann, A.F. and Borgström, B. (1964)
The intraluminal phase of fat digestion in man: the lipid content of the micellar and oil phases of intestinal content obtained during fat digestion and absorption.
Journal of Clinical Investigation, 43, 247-257

Hofmann, A.F. and Hoffman, N.E. (1974)
Measurement of bile acid kinetics by isotope dilution in man.
Gastroenterology, 67, 514-523

Hofmann, A.F. and Poley, R.J. (1969)
Cholestyramine treatment of diarrhoea associated with ileal resection.
New England Journal of Medicine, 281, 397-402

Holt, P.R. (1972)
The roles of bile acids during the process of normal fat and cholesterol absorption.
Archives of Internal Medicine, 130, 574-583

Hughes, J.A., Bates, T. and Dowling, R.H. (1973)
Cholecystokinin and secretion prevent the intestinal mucosal hypoplasia of total parenteral nutrition in the dog.
Gastroenterology, 75, 31-41

Hutson, J.J. (1976)
Intestinal ultrastructure after prolonged use of an elemental diet.
Strahlentherapie, 151, 541-543

Imondi, A.R., Balis, M.E. and Lipkin, M. (1969)
Changes in enzyme levels accompanying differentiation of intestinal epithelial cells.
Experimental Cell Research, 58, 325-330

Jacobs, P., Cotnwell, T.A. and Charlton, C.N. (1966)
Intestinal iron transport: studies using a loop of the gut with an artificial circulation.
American Journal of Physiology, 210, 694-700

Jacobs, L.R., Taylor, B.R. and Dowling, R.H. (1975)
Effect of luminal nutrition on the intestinal adaptation following Thiry-Vella by-pass in the dog.
Clinical Science and Molecular Medicine, 49, 267p (Abstract)

Johnson, L.R., Copeland, E.M., Dudrick, S.J., Lichtenberger, L.M and Castro, G.A. (1975)
Structural and hormonal alterations in the gastrointestinal tract of parenterally fed rats.
Gastroenterology, 68, 1177-1183

Jones, D.J., Melchior, G.W. and Reeves, A.J.W. (1976)
Quantitative analysis of individual bile acids by gas-liquid chromatography: an improved method.
Journal of Lipid Research, 17, 273-277

Kellog, T.F., Knight, L. and Mostmann, B.S. (1970)
Effect of bile acid deconjugation on the faecal excretion of steroids.
Journal of Lipid Research, 11, 362-366

Kellog, T.F. and Mostmann, B.S. (1969)
Faecal neutral steroids and bile acids from germ-free rats.
Journal of Lipid Research, 10, 495-503

Kelly, G.A. and Mahrwold, D.L. (1976)
Pancreatic secretion in response to an elemental diet and intravenous hyperalimentation.
Surgery, Gynecology, Obstetrics, 143, 87-91

Kershaw, J.G., Keane, K.D. and Wiseman, G. (1969)
The effect of semi-starvation on absorption by the rat small intestine in vitro and in vivo.
Journal of Physiology, 152, 182-190

Kim, Y.S. (1977)
Intestinal mucosal hydrolysis of proteins and peptides.
Ciba Foundation Symposium, 50, 151-176

Kim, Y.S., Kim, Y.W. and Gleisenger, M.H. (1974)
Studies on the properties of peptide hydrolases in the brush border and soluble fractions of small intestinal mucosa of rat and man.
Biochimica et Biophysica Acta, 370, 233-246

Lack, L. and Weiner, I.M. (1961)
In vitro absorption of bile salts by small intestine of rats and guinea pigs.
American Journal of Physiology, 200, 313-317

Lack, L. and Weiner, I.M. (1966)
Intestinal bile salt transport:structure activity relationships.
American Journal of Physiology, 210, 1142-1152

Langley, R. (1963)
Practical Statistics pp 166-173. London:Pan

Larralde, J., Fernandez-Otero, P. and Gonzalez, M. (1966)
Increased active transport of glucose through the intestine during pregnancy.
Nature, 209,1356-1357

Leblond, C.P. and Walker, B.E. (1956)
Renewal of cell populations.
Physiological Reviews, 36, 255-276

Lee, J.J. and Hermann, R.G. (1963)
Sucrose diet and biliary cholate excretion in rats: with note on procedure for cholate determination.
Archives Internationales de Pharmacodynamie et de Therapie, 141, 591-594

Leese, H.J. (1974)
Glucose accumulation by rat small intestine during absorption in vivo.
Nature, 251, 512-513

Leese, H.J., Prendergast, J. and Read, B.S. (1976)
The effect of theophylline on glucose and fluid transport across the rat jejunum.
Biochemical Society Transactions, 4, 272-274

Levenson, S.M., Kan, D., Grube, D., Crowley, L., Jaffe, B.R. Nakao, K., Geever, E. F. and Seifter, E. (1971)
Hemolytic anemia and pancreatic acinar atrophy and fibrosis conditioned by elemental liquid diets and the ordinary intestinal flora.
Annals of Surgery, 174, 469-520

Levine, G.M., Deren, J.J., Steiner, E. and Zinno, R. (1974)
Role of oral intake in maintenance of gut mass and disaccharidase activity.
Gastroenterology, 69, 975-982

Lewis, D.D. and Fordtran, J.S. (1975)
Effect of perfusion rate on absorption, surface area, unstirred water layer thickness, permeability and intestinal pressure in the rat ileum in vivo.
Gastroenterology, 68, 1509-1516

Lewis, D. and Goebach, S. (1972)
Modification of bile acids by intestinal bacteria.
Archives of Internal Medicine, 130, 545-549

Lindstedt, S. and Norman, A. (1956)
The turnover of bile acids in the rat.
Acta Physiologica Scandinavica 38, 121-123

Lipkin, M. (1973)
Proliferation and differentiation of gastrointestinal cells.
Physiological Reviews, 53, 891-915

Lipkin, M., Sherlock, P. and Bell, B. (1963)
Cell proliferation kinetics in the gastrointestinal tract of man.
II Cell renewal in the stomach, ileum, colon and rectum.
Gastroenterology, 45, 721-729

Low-Beer, T.S. and Heaton, K.W. (1973)
The effect of coeliac disease upon bile salts.
Gut, 14, 204-208

Low-Beer, T.S., Pomare, E.W. and Morris, J.S. (1972)
Control of bile salt synthesis.
Nature (New Biology), 238, 215-216

Low-Beer, T.S., Wilkins, R.M., Lack, L. and Tyor, M.P. (1974)
Effect of one meal on the enterohepatic circulation of bile salts.
Gastroenterology, 67, 490-497

MacDonald, W.C., Trier, J.S. and Everett, N.B. (1964)
Cell proliferation and migration in the stomach, duodenum, and rectum of man: autoradiographic studies.
Gastroenterology, 46, 405-417

Madsen, D., Beaver, J., Zhang, L., Bruckner-Kardoss, E. and Wostmann, B.S. (1976)
Analysis of bile acids in conventional and germ-free rats.
Journal of Lipid Research, 17, 107-111

Madsen, D., Zhang, L. and Wostmann, B.S. (1975)
Muricholate, tertiary bile acid of the Wistar rat.
Proceedings of the Indiana Academy of Science, 84, 416-420

Makita, M. and Wells, W.W. (1963)
Quantitative analysis of faecal bile acids by gas-liquid chromatography.
Analytical Biochemistry, 5, 523-530

Matthews, D.M., and Lester, L. (1965)
Absorption of protein digestion products: a review.
Gut, 6, 111-126

Matthews, D.M., Lis, I.T., Zheng, E. and Crampton, R.F. (1969)
Observations on the intestinal absorption of some oligopeptides of methionine and glycine in the rat.
Clinical Science, 37, 751-764

McArdle, A.H., Brown, R.A., Schave, V., Rivilis, J. and Thomson, A.B. (1972)
Alterations in gastric and pancreatic secretion induced by feeding of an elemental diet.
Archives des Maladies de l'Appareil Digestif et de la Nutrition, 61, 115c (Abstract)

Meihoff, W.E. and Kern, F. (1963)
Bile salt malabsorption in regional ileitis, ileal resection and mannitol-induced diarrhoea.
Journal of Clinical Investigation, 47, 261-267

Mickelsen, O. (1962)
Nutrition - germ-free animal research.
Annual Review of Biochemistry, 31, 515-548

Miettinen, T.A., and Tarmila, S. (1977)
Effect of pectin on serum cholesterol, faecal bile acids and biliary lipids in normolipidemic and hyperlipidemic individuals.
Clinica Chimica Acta, 79, 471-477

Mitropoulous, K.A. and Myant, N.B. (1967)
The formation of litocholic acid, chenoleoxycholic acid and α and β-muricholic acids from cholesterol incubated with rat liver mitochondria.
Biochemical Journal, 103, 472-479

Mekhjian, H.S., Phillips, S.F. and Hofmann, A.F. (1971)
Colonic secretion of water and electrolytes induced by bile acids: perfusion studies in man.
Journal of Clinical Investigation, 50, 1569-1577

Miller, J.L. and Taboada, J.J. (1975)
Clinical experience with an elemental diet.
American Journal of Clinical Nutrition, 28, 46-50

Mosbach, E.H. (1972)
Hepatic synthesis of bile acids. Biochemical steps and mechanisms of rate control.
Archives of Internal Medicine, 130, 478-487

Myant, N.B. and Mitropoulous, K.A. (1977)
Cholesterol 7α hydroxylase.
Journal of Lipid Research, 18, 135-153

Nalin, D.R., Jash, T.A., Rahman, M. and Yunus, M.D. (1970)
Effects of glycine and glucose on sodium and water absorption in patients with cholera.
Gut, 11, 768-772

Newey, H. and Smyth, D.H. (1962)
Cellular mechanisms in intestinal transfer of amino acids.
Journal of Physiology, 164, 527-551

Norman, A. and Sjovall, J. (1958)
On the transformation and enterohepatic circulation of cholic acid in the rat.
Journal of Biological Chemistry, 233, 872-885

Nort field, T.J. and McColl, I. (1973)
Postprandial concentrations of free and conjugated bile acids down the length of the normal human intestine.
Gut, 14, 513-518

Ockner, R.K., Hughes, F.B. and Isselbacher, K.J. (1969)
Very low density lipoproteins in intestinal lymph.
Journal of Clinical Investigation, 48, 2367-2373

Oda, T. and Seki, S. (1965)
Molecular structure and biochemical function of the microvilli membrane of intestinal epithelial cells, with special emphasis on the elementary particles.
Journal of Electron Microscopy, 14, 210-217

Olsen, W.A. and Ingelfinger, F.J. (1968)
The role of sodium in intestinal glucose absorption in man.
Journal of Clinical Investigation, 47, 1133-1142

O'Maille, E.R.L., Richards, T.G. and Short, A.H. (1967)
The influence of conjugation of cholic acid on its uptake and secretion: hepatic extraction of taurocholate and cholate in the dog.
Journal of Physiology, 189, 337-350

Pageau, R. (1975)
Effects of feeding and elemental diet during abdominal irradiation
Strahlentherapie, 149, 313-323

Payler, D.K., Pomare, E.W., Heaton, K.W. and Harvey, R.F. (1975)
The effect of wheat bran on intestinal transit.
Gut, 16, 209-213

Perrault, J., Devroede, G. and Bounous, G. (1973)
Effects of an elemental diet in healthy volunteers.
Gastroenterology, 64, 569-576

Phillips, S.F. and Summerskill, W.H.J. (1966)
Occlusion of the jejunum for intestinal perfusion in man.
Mayo Clinic Proceedings, 41, 224-231

Playoust, M.R., Lack, L. and Weiner, I.M. (1965)
Effect of intestinal resection on bile salt absorption in dogs.
American Journal of Physiology, 208, 363-369

Pomare, E.W. and Heaton, K.W. (1973)
Effect of cholecystectomy on bile salt metabolism.
Gut, 14, 753-762

Portman, O.W. (1960)
Nutritional influences on the metabolism of bile acids.
American Journal of Clinical Nutrition, 8, 462-470

Portman, O.W., Hahn, G.V. and Wysocki, A.P. (1955)
Bile acid excretion by rat: nutritional effects.
Archives of Biochemistry and Biophysics, 59, 224-252

Portman, O.W. and Murphy, P. (1953)
Excretion of bile acids and β-hydroxysterols by rats.
Archives of Biochemistry and Biophysics, 76, 367-376

Ragins, A., Levenson, S.M., Signer, R., Stamford, W. and Seifter, E. (1973)
Intrajejunal administration of an elemental diet at neutral pH avoids pancreatic stimulation: studies in dog and man.
American Journal of Surgery, 126, 606-614

Raicht, R.F., Deschner, E. and Salen, G. (1975)
Bile acids regulate intestinal cell turnover.
Gastroenterology, 68, 979 (Abstract)

Rasmara, G.B., Henderson, L.I. and Elvehjem, C.A. (1949)
Purified amino acids as a source of nitrogen for the growing rat.
Journal of Nutrition, 38, 177-194

Reddy, B.S., Pleasants, J.R. and Wostmann, B.S. (1968)
Effect of dietary carbohydrate on intestinal disaccharidases in germ-free and conventional rats.
Journal of Nutrition, 95, 413-419

Rijke, R.P.J., Hanson, W.R., Plaiser, H.M., and Osborne, J.W. (1976)
The effect of ischemic villus cell damage on crypt cell proliferation in the small intestine. Evidence for a feed-back control mechanism.
Gastroenterology, 71, 736-742

Roberts, P.J.P. and Whelan, W.J. (1960)
The mechanism of carbohydrase action. 5. Action of human salivary α-amylase on amylopectin and glycogen.
Biochemical Journal, 76, 246-253

Robinson, J.W.L. (1972)
Intestinal malabsorption in the experimental animal.
Gut, 13, 938-945

Rose, W.C. (1949)
Amino acid requirement of man.
Federation Proceedings, 8, 546-552

Rose, W.C. (1957)
The amino acid requirements of adult man.
Nutritional Abstract Reviews, 27, 631-647

Rose, W.C., Oesterling, M.J. and Womack, M.J. (1948)
Comparative growth on diets containing ten and nineteen amino acids, with further observations upon the role of glutamic and aspartic acids.
Journal of Biological Chemistry, 176, 753-762

Rose, W.C. and Wixon, R.L. (1955)
Amino acid requirements of man. XVI The role of nitrogen intake.
Journal of Biological Chemistry, 217, 997-1004

Rose, W.C., Wixon, R.L., Lockhart, E. and Lambert, G.F. (1955)
Amino acid requirements of man. XV The valine requirement: summary and final observations.
Journal of Biological Chemistry, 217, 987-995

Rulo, L.P. and Rosenberg, I.H. (1973)
Chronic glucagon administration enhances intestinal transport in the rat.
Proceedings of the Society for Experimental Biology and Medicine, 142, 521-525

Russell, R.I. (1975)
Elemental Diets
Gut, 16, 63-79

Russell, R.I., Allan, J.G., Gerskowitch, V.P. and Robertson, J.W.K. (1972) A study by perfusion techniques of the absorption abnormalities in the jejunum in adult coeliac disease.
Clinical Science, 42, 735-741

Sarda, L. and Desnuelle, P. (1953)
Action de la lipase pancréatique SUR, les esters en émulsion.
Biochimica et Biophysica Acta, 30, 513-521

Schedl, H.P. (1966)
Use of PAG and phenol red as unabsorbed indicators for intestinal absorption studies in man.
Gut, 7, 159-163

Schedl, H.P. and Clifton, J.A. (1963)
Solute and water absorption by the human small intestine.
Nature, 199, 1264-1267

Scherstén, T. (1967)
The synthesis of cholic acid conjugates in human liver.
Acta Chirurgica Scandinavica, 373, 1-33

Schiff, E.R., Small, M.J. and Dietschy, J.M. (1972)
Characterization of kinetics of passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat.
Journal of Clinical Investigation, 51, 1351-1362

Schmid, R.J., Phillips, S.F. and Summerskill, W.H.J. (1969)
Jejunal secretion of electrolytes and water in non-tropical sprue.
Journal of Laboratory and Clinical Medicine, 73, 772-733

Schultze, M.O. (1956)
Reproduction of rats fed protein-free rations.
Journal of Nutrition, 60, 35-45

Senior, J.R. and Isselbacher, K.J. (1963)
Demonstration of an intestinal mono-glyceride lipase: an enzyme with a possible role in the intracellular completion of fat digestion.
Journal of Clinical Investigation, 42, 137-145

Shefer, S., Hauser, S., Bekersky, I. and Mosbach, E.H. (1969)
Feedback regulation of bile acid biosynthesis in the rat.
Journal of Lipid Research, 10, 646-655

Sneff, M.F. and Smyth, D.H. (1955)
An apparatus for the study of in vivo intestinal absorption in the rat.
Journal of Physiology, 128, 67 p (Abstract)

Siegel, S. (1956)
Non-parametric statistics, pp 116-126. Kogakusha:McGraw-Hill

Silk, D.B.A, Marrs, T.C., Addison, J.M., Burston, D., Clark, M.L. and Matthews, D.M. (1975)
Absorption of amino acids from an amino acid mixture simulating casein a tryptic hydrolysate of casein in man.
Clinical Science and Molecular Medicine, 45, 715-719

Simko, V. and Linsheer, W.G. (1976)
Absorption of different elemental diets in a short bowel syndrome lasting 15 years.
American Journal of Digestive Diseases, 21, 419-425

Simmonds, W.J., Hofmann, A.F. and Theobor, D. (1967)
Absorption of cholesterol from a micellar solution: intestinal perfusion studies in man.
Journal of Clinical Investigation, 46, 874-880

Siperstein, M.D., Chaikoff, I.L. and Reinhardt, W.O. (1952)
^{131}I cholesterol: Obligatory function of bile in intestinal absorption of cholesterol.
Journal of Biological Chemistry, 198, 111-114

Sjovall, J. (1960)
Bile acids in man under normal and pathological conditions.
Clinica Chimica Acta, 5, 33-41

Skou, J.C. (1965)
Enzymatic basis for active transport of sodium and potassium across cell membranes.
Physiological Reviews, 45, 596-617

Sladen, G.E. and Dawson, A.M. (1968)
An evaluation of perfusion techniques in the study of water and electrolyte absorption in man: the problem of endogenous secretions.
Gut, 9, 530-535

Sladen, G.E. and Dawson, A.M. (1969)
Inter-relationships between the absorption of glucose, sodium and water by the normal human jejunum.
Clinical Science, 36, 119-132

Spector, M.H., Levine, G.M. and Deren, J.J. (1977)
Direct and indirect effects of dextrose and amino acids on gut mass.
Gastroenterology, 72, 706-710

Stanley, I.L., Paul, J., Gaske, D. and Murphy, J. (1973)
Comparative effects of cholestyramine, metamucil and cellulose on faecal bile salt excretion in man.
Gastroenterology, 65, 389-394

Stephens, A.V. and Randall, H.T.
Use of concentrated, balanced, liquid elemental diet for nutritional management of catabolic states.
Annals of Surgery, 170, 642-647

Stewart, J.S. (1974)
Clinical and morphologic response to gluten withdrawal.
In Clinics in Gastroenterology, Coeliac Disease
Ed. Cooke, W.T. and Asquith, P. Vol 3 (1), Ch. 7 London:Saunders

Strand, O. (1965)
Effects of D-and-L-tri-iodothyronine and of propylthiouracil on the production of bile acids in the rat.
Journal of Lipid Research, 4, 305-311

Thomas, P.J., Hsia, D.L., Matschiner, J.T., Doisy, E.A. Elliott, W.H. and Thayer, S.A. and Doisy, E.A. (1964)
Bile acids. XIX Metabolism of lithocholic-24-^{14}C in the rat.
Journal of Biological Chemistry, 239, 102-105

Thompson, W.R., Stephens, A.V., Randall, H.T. and Bowen, J.R. (1969)
Use of the 'Space-Diet' in the management of a patient with extreme short bowel syndrome.
American Journal of Surgery, 117, 449-459

Toskes, P.P., Giannella, R.A., Jervis, H.R., Rout, W.R. and Takeuchi, A. (1975)
Small intestinal mucosal injury in the experimental blind loop syndrome.
Gastroenterology, 68, 1193-1203

Vantrappen, G., Ghoos, Y., Rutgeerts, P. and Janssens, J. (1977)
Bile acid studies in uncomplicated Crohn's disease.
Gut, 18, 730-735

Vilon, N., Hecketsweiler, P., Dutel, J. and Bernier, J.J. (1978)
Effect of continuous jejunal perfusion of elemental and complex nutritional solutions on pancreatic enzyme secretion in human subjects.
Gut, 19, 191-198

Voitk, A.J., Echave, V., Feller, J.F., Brown, R.A. and Gurd, F.N. (1973a)
Experience with elemental diet in the treatment of inflammatory bowel disease.
Archives of Surgery, 107, 329-333

Voitk, A.J., Echave, V., Brown, R.A., McArdle, A.H. and Gurd, F.N. (1973b)
Elemental diet in the treatment of fistulas of the alimentary tract.
Surgery, Gynecology, Obstetrics, 137, 63-72

Voitk, A.J., Echave, V., Brown, R.A. and Gurd, F.N. (1973c)
Use of elemental diet during the adaptive phase of short gut syndrome.
Gastroenterology, 65, 417-426

Voitk, A.J., Brown, R.A., Mohave, V., McArdle, A.H., Gurd, F.N. and Thomson, A.G. (1973)
Use of an elemental diet in the treatment of complicated pancreatitis.
American Journal of Surgery, 125, 223-227

Watson, A.J. and Wright, R.A. (1974)
Morphology and cell kinetics of the jejunal mucosa in untreated patients.
In Clinics in Gastroenterology. Coeliac Disease Ed. Cooke, W.T., and Asquith, P. Vol 3(1), Ch 2. London:Saunders.

Weser, E. and Hernandez, B.A. (1971)
Studies of small bowel adaptation after intestinal resection in the rat.
Gastroenterology, 60, 69-75

Weser, E., Heller, R. and Tawil, N. (1977)
Stimulation of mucosal growth in the rat ileum by bile and pancreatic secretions after jejunal resection.
Gastroenterology, 73, 524-529

Wheeler, H.O. (1971)
Concentrating function of the gallbladder.
American Journal of Medicine, 51, 533-595

Wheeler, H.O. (1972)
Secretion of bile acids by the liver and their role in the formation of hepatic bile.
Archives of Internal Medicine, 130, 533-541

Wiggins, H.S. (1967)
Simple method for determining trypsin.
Gut, 8, 415-416

Williamson, R., Bauer, J., Ross, J. and Malt, R. (1973)
Proximal enterectomy stimulates distal hyperplasia more than bypass or pancreaticobiliary diversion.
Gastroenterology, 74, 16-23

Wingate, D.L., Sandberg, R.J. and Phillips, S.F. (1972)
A comparison of stable and ^{14}C labelled polyethylene glycol as volume indicators in the human jejunum.
Gut, 13, 812-815

Winitz, M., Birnbaum, S.M. and Greenstein, J.P. (1957a)
Quantitative studies with water soluble, chemically defined diets. IV Influence of various carbohydrates on growth, with special reference to D-glucosamine.
Archives of Biochemistry and Biophysics, 72, 437-477

Winitz, M., Greenstein, J.P. and Birnbaum, S.M. (1957b)
Quantitative studies with water soluble, chemically defined diets. V Role of isomeric arginines in growth.
Archives of Biochemistry and Biophysics, 72, 448-456

Winitz, M., Birnbaum, J.M., Sugimura, T. and Otey, M.C. (1960)
Quantitative nutritional and in vivo metabolic studies with water soluble chemically defined diets.
In Amino Acids, Proteins and Cancer Biochemistry, ed. Bisall, T. pp 9-41 New York:Academic Press

Winitz, M., Graff, J. and Seedman, D.A. (1964)
Effect of dietary carbohydrate on serum cholesterol levels.
Archives of Biochemistry, 108, 576-579

Winitz, M., Graff, J., Gallagher, N., Markin, A. and Seedman, D.A. (1965)
Evaluation of chemical diets as nutrition for man in space.
Nature, 205, 741-743

Winitz, M., Adams, R.F., Seedman, D.A., and Graff, J. (1966)
Regulation of intestinal flora patterns with chemical diets.
Federation Proceedings, 25, 343 (Abstract)

Winitz, M., Seedman, D.A. and Graff, J. (1970a)
Studies in metabolic nutrition employing chemically defined diets.
I Extended feeding to normal adult males.
American Journal of Clinical Nutrition, 23, 525-545

Winitz, M., Adams, R.F., Seedman, D.A., Davis, P.N., Jayko, L.G. and Hamilton, J.A. (1970b)
Studies in metabolic nutrition employing chemically defined diets.
II Effects on gut microflora population.
American Journal of Clinical Nutrition, 23, 546-559

Wiseman, G. (1955)
Absorption of amino acids using an in vitro technique.
Journal of Physiology, 129, 63-72

Wiseman, G., Deane, M.D. and Ghadially, F.N. (1959)
Effect of sarcoma 180_3 on intestinal active absorption of glucose and L-histidine.
British Journal of Cancer, 13, 232-237

Yardley, J.H., Hayless, T.M., Norton, J.H. and Hendrix, T.R. (1962)
Celiac disease. A study of the jejunal epithelium before and after a gluten-free diet.
New England Journal of Medicine, 267, 1173-1179

Young, L.A., Violetti, L.A. and Weber, F. (1973)
The effect of defined formula diets on mucosal mass of the small bowel in the rat.
Gastroenterology, 74, 1115 (Abstract)